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(54) **COMPOSITION FOR PRODUCING TAGATOSE FROM FRUCTOSE-6-PHOSPHATE AND METHOD OF PRODUCING TAGATOSE FROM FRUCTOSE-6-PHOSPHATE USING THE SAME**

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C12N 9/90	(2006.01)
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C12P 19/24	(2006.01)
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CPC ***C12N 9/88*** (2013.01); ***C12N 9/1051*** (2013.01); ***C12N 9/1205*** (2013.01); ***C12N 9/90*** (2013.01); ***C12P 19/02*** (2013.01); ***C12P 19/24*** (2013.01); ***C12Y 204/01025*** (2013.01); ***C12Y 207/01002*** (2013.01); ***C12Y 207/01144*** (2013.01); ***C12Y 401/0204*** (2013.01); ***C12Y 503/01009*** (2013.01); ***C12Y 504/02002*** (2013.01)

(58) **Field of Classification Search**
None
See application file for complete search history.

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(57) **ABSTRACT**

The present disclosure relates to a composition for producing tagatose, comprising fructose-6-phosphate-4-epimerase, and a method of producing tagatose using the same.

11 Claims, 10 Drawing Sheets

Specification includes a Sequence Listing.

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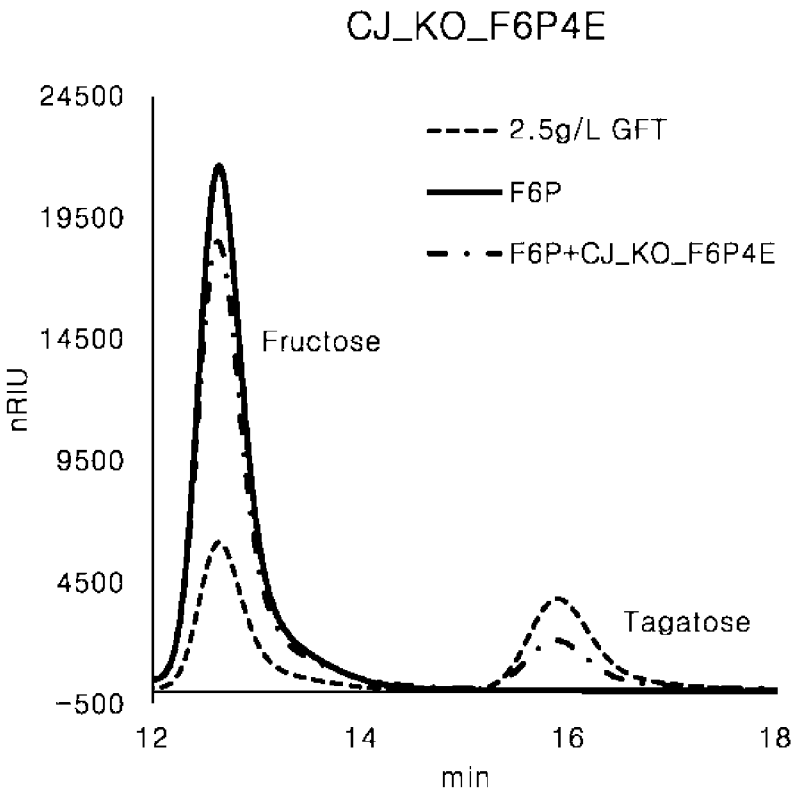
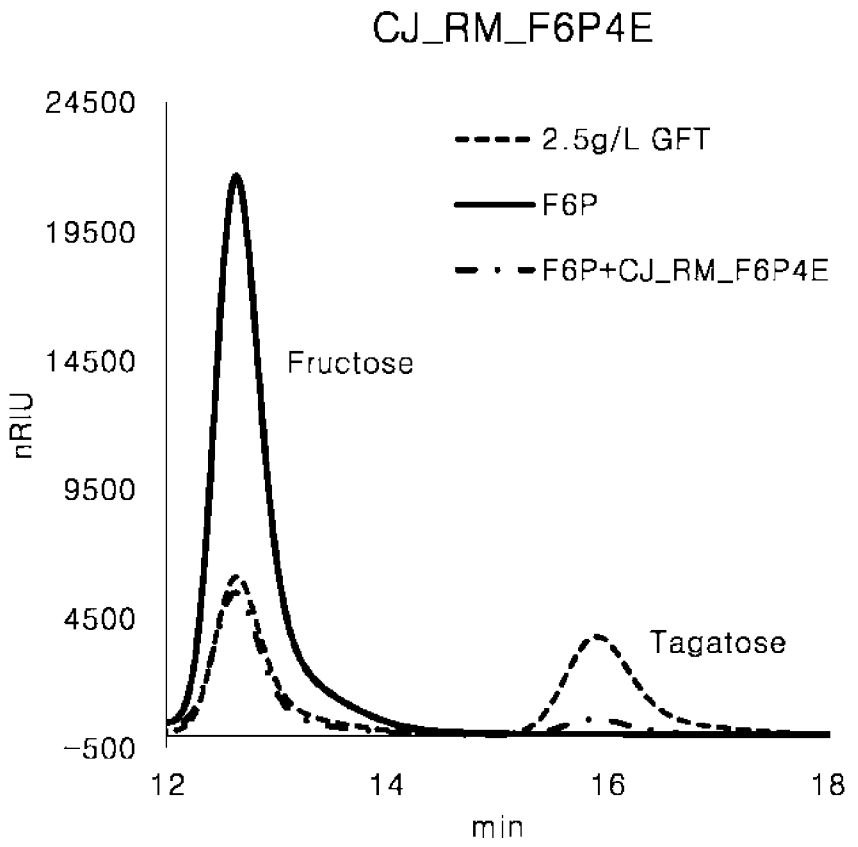
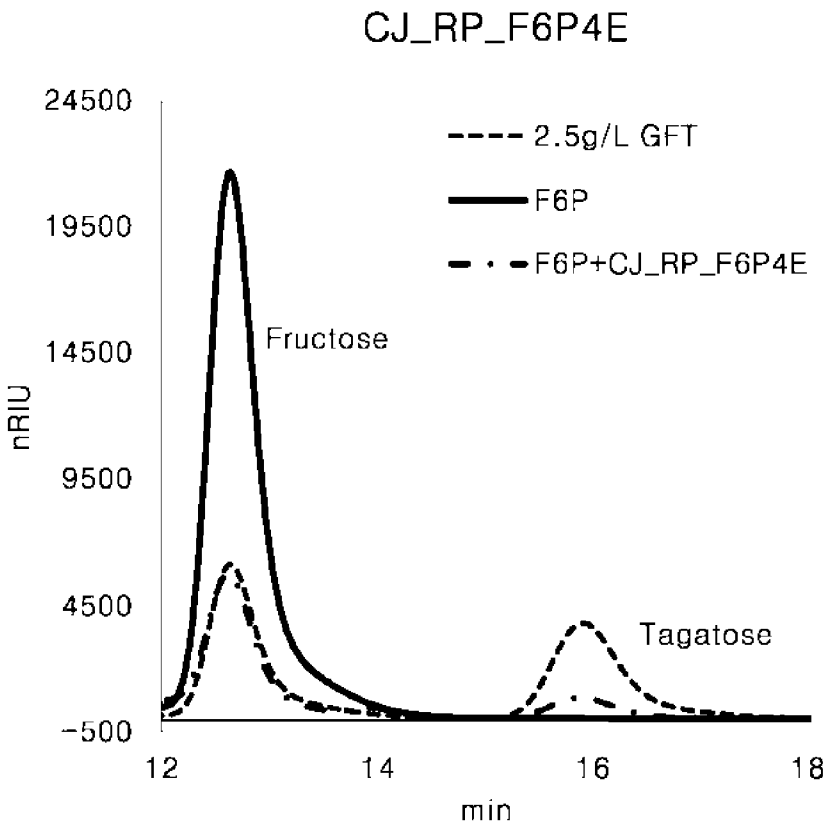
[FIG. 1a]


CJ_KO_F6P4E
24500
19500
14500
9500
4500
-500
nRIU
2.5g/L GFT
F6P
F6P+CJ_KO_F6P4E
Fructose
Tagatose
12
14
16
18
min

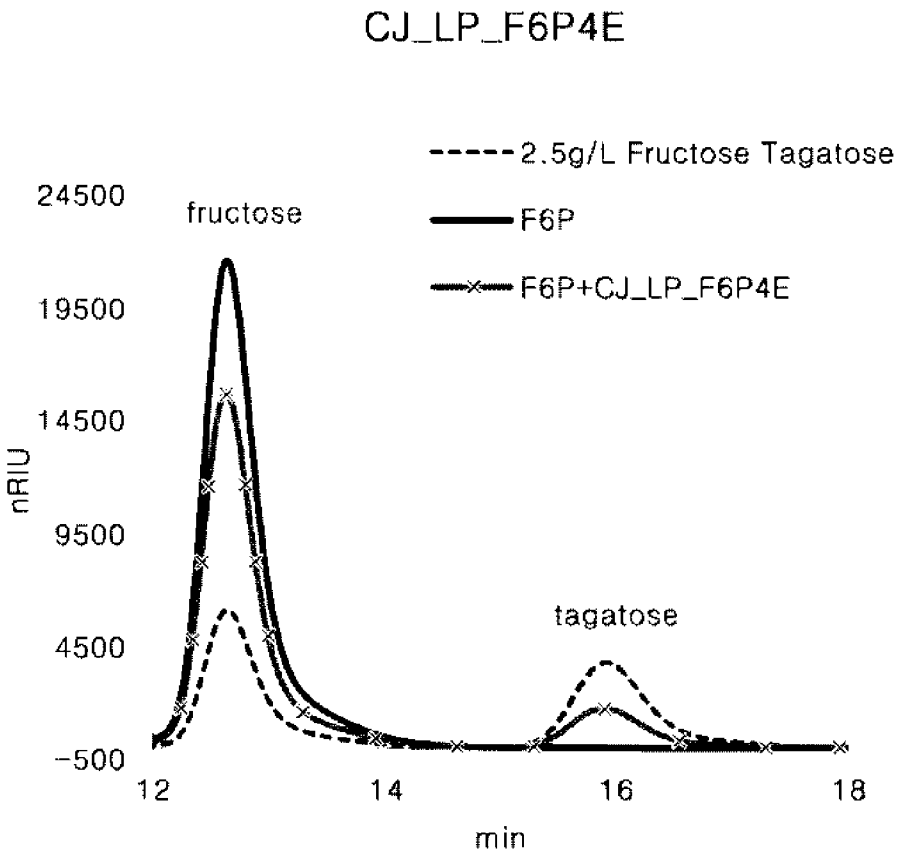
[FIG. 1b]


CJ_RM_F6P4E
24500
19500
14500
9500
4500
−500
nRIU
2.5g/L GFT
F6P
F6P+CJ_RM_F6P4E
Fructose
Tagatose
12
14
16
18
min

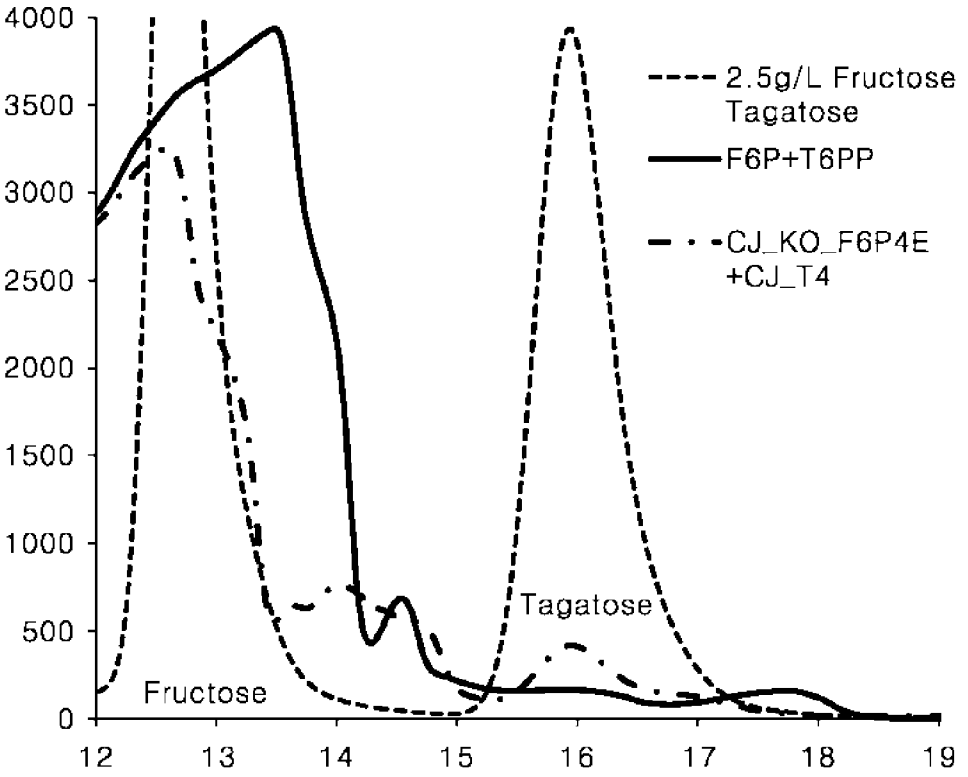
[FIG. 1c]


CJ_RP_F6P4E
24500
19500
14500
9500
4500
-500
nRIU
2.5g/L GFT
F6P
F6P+CJ_RP_F6P4E
Fructose
Tagatose
12
14
16
18
min

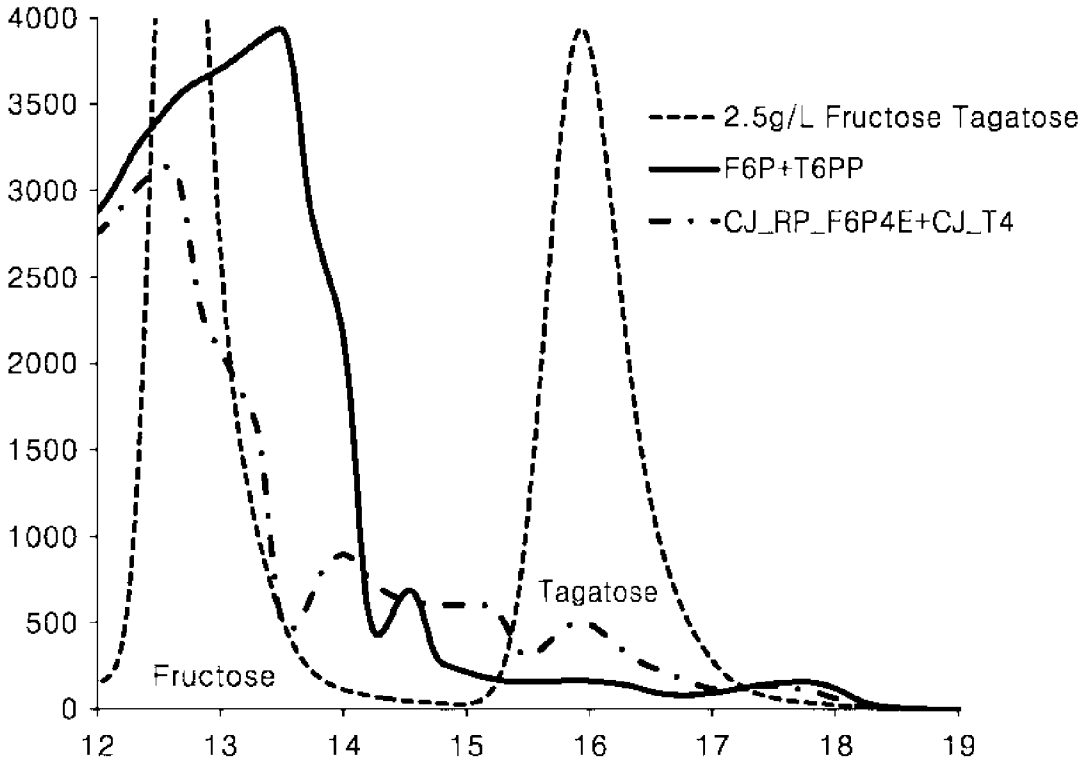
[FIG. 1d]


CJ_LP_F6P4E
2.5g/L Fructose Tagatose
F6P
F6P+CJ_LP_F6P4E
fructose
tagatose
24500
19500
14500
9500
4500
-500
nRIU
12
14
16
18
min

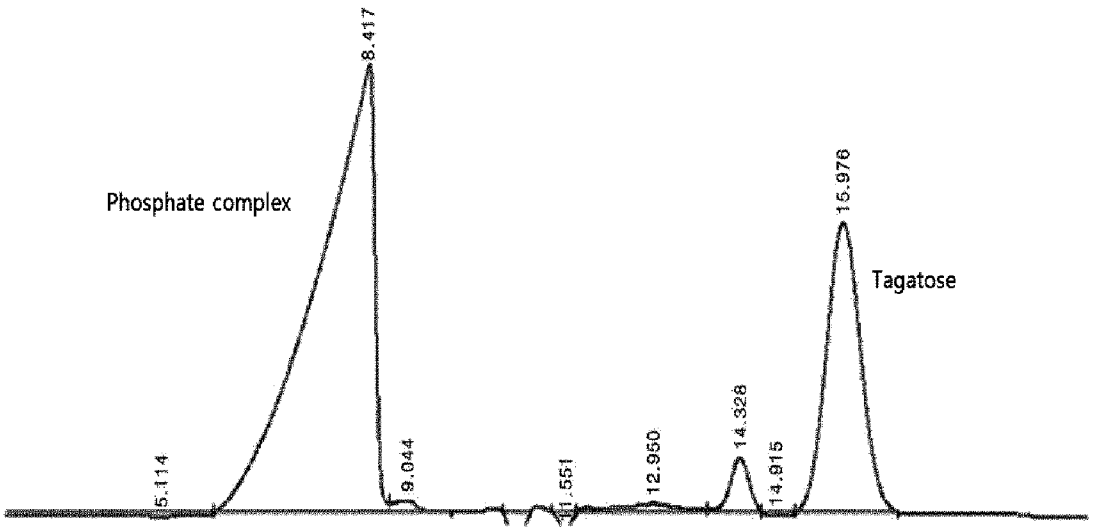
[FIG. 2a]


4000
3500
3000
2500
2000
1500
1000
500
0
12
13
14
15
16
17
18
19
2.5g/L Fructose Tagatose
F6P+T6PP
CJ_KO_F6P4E +CJ_T4
Fructose
Tagatose

[FIG. 2b]


4000
3500
3000
2500
2000
1500
1000
500
0
12
13
14
15
16
17
18
19
2.5g/L Fructose Tagatose
F6P+T6PP
CJ_RP_F6P4E+CJ_T4
Fructose
Tagatose

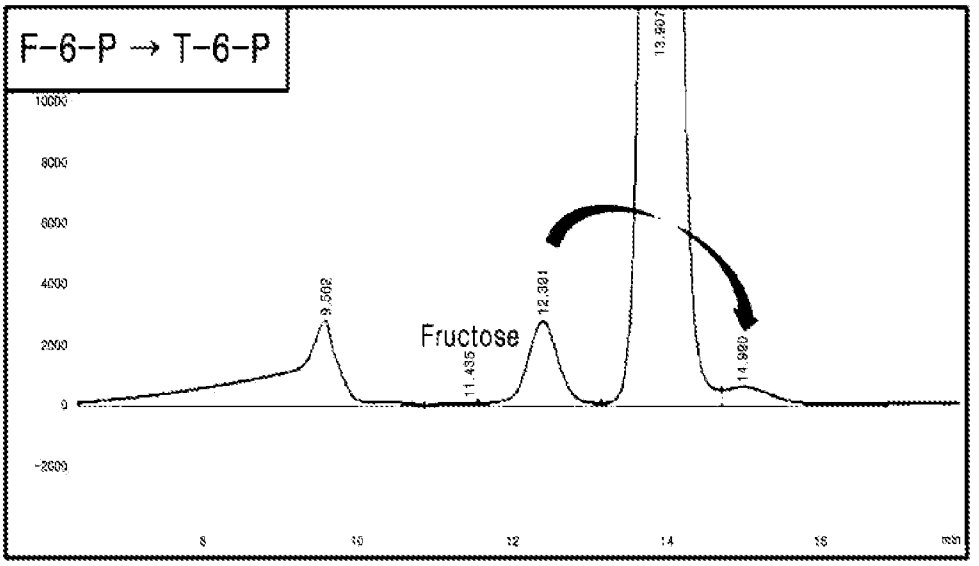
[FIG. 3]


Phosphate complex
8.417
15.976
Tagatose
5.114
9.044
11.551
12.950
14.328
14.915

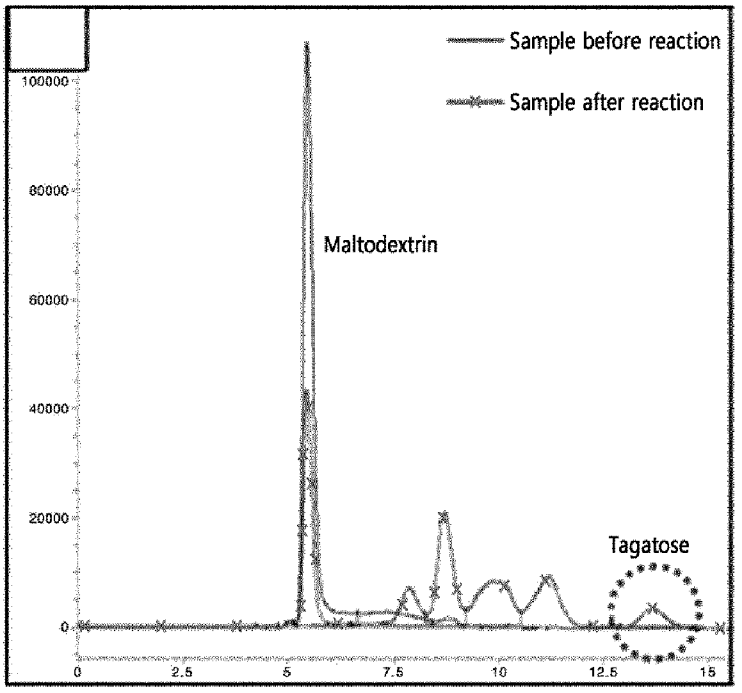
[FIG. 4]

M: Protein size ladder(10~250kDa)
CT1: *E. coli* BL21(DE3)/pET21a-CJ_ct1
TN1: *E. coli* BL21(DE3)/pET21a-CJ_tn1
CT2: *E. coli* BL21(DE3)/pET21a-CJ_ct2
TD1: *E. coli* BL21(DE3)/pBT7-C-His-CJ_td1
AN1: *E. coli* BL21(DE3)/pBT7-C-His-an1
T4: *E. coli* BL21(DE3)/pET21a-CJ_t4

[FIG. 5]



[FIG. 6]


Sample before reaction
Sample after reaction
Maltodextrin
Tagatose
100000
80000
60000
40000
20000
0
0
2.5
5
7.5
10
12.5
15

COMPOSITION FOR PRODUCING TAGATOSE FROM FRUCTOSE-6-PHOSPHATE AND METHOD OF PRODUCING TAGATOSE FROM FRUCTOSE-6-PHOSPHATE USING THE SAME

CROSS REFERENCE TO RELATED APPLICATIONS

This application is the National Stage of International Application No. PCT/KR2018/003748, filed Mar. 30, 2018, which claims the benefit of Korean Patent Application No. 10-2017-0042165, filed Mar. 31, 2017, the contents of which are incorporated by reference in their entireties.

BACKGROUND or THE INVENTION

1. Field of the Invention

The present disclosure relates to a composition for producing tagatose-6-phosphate, comprising fructose-6-phosphate 4-epimerase, and a method of producing tagatose using the same.

2. Description of the Related Art

Conventional methods of producing tagatose include a chemical method (a catalytic reaction) and a biological method (an isomerization enzyme reaction) of using galactose as a main raw material (see Korean Patent No. 10-0964091). However, the price of lactose which is a basic raw material of galactose used as a main raw material in the known production methods is unstable, depending on produced amounts, supply, and demand of raw milk and lactose in global markets, etc. Thus, there is a limitation in the stable supply thereof. To overcome the problem of the conventional methods of producing tagatose, methods of producing tagatose from D-fructose having a low price and steady supply using hexuronate C4-epimerase have been reported (2011. Appl Biochem Biotechnol. 163:444-451; Korean Patent No. 10-1550796). However, there is a limitation in that the isomerization has a low conversion rate.

Tagatose-bisphosphate aldolase (EC 4.1.2.40) is known to produce glycerone phosphate and D-glyceraldehyde 3-diphosphate from D-tagatose 1,6-bisphosphate as a substrate, as in the following [Reaction Scheme 1], and to participate in a galactose metabolism. However, there have been no studies regarding whether the tagatose-bisphosphate aldolase has activity to convert fructose-6-phosphate into tagatose-6-phosphate.

D-tagatose 1,6-bisphosphate <=>glycerone phosphate+D-glyceraldehyde 3-diphosphate [Reaction Scheme 1]

Under this background, the present inventors have conducted extensive studies to develop an enzyme which may be used in the production of tagatose, and as a result, they found that tagatose-bisphosphate aldolase (EC 4.1.2.40) as the ability to convert glucose-6-phosphate into tagatose-6-phosphate, thereby completing the present disclosure.

Accordingly, glucose or starch may be used as a raw material to sequentially produce glucose-1-phosphate and glucose-6-phosphate, and then tagatose-bisphosphate aldolase of the present disclosure may be used to convert glucose-6-phosphate into tagatose-6-phosphate, and tagatose-6-phosphate phosphatase which performs an irreversible reaction pathway may be used to produce tagatose while remarkably increasing a conversion rate of glucose or starch into tagatose.

SUMMARY OF THE INVENTION

An object of the present disclosure is to provide a composition useful for the production of tagatose-6-phosphate, comprising tagatose-bisphosphate aldolase, a microorganism expressing the tagatose-bisphosphate aldolase, or a culture of the microorganism.

Another object of the present disclosure is to provide a composition useful for the production of tagatose, comprising tagatose-bisphosphate aldolase, a microorganism expressing the tagatose-bisphosphate aldolase, or a culture of the microorganism; and tagatose-6-phosphate phosphatase, the microorganism expressing the tagatose-6-phosphate phosphatase, or a culture of the microorganism.

Another object of the present disclosure is to provide a method of producing tagatose, comprising converting fructose-6-phosphate into tagatose-6-phosphate by contacting fructose-6-phosphate with tagatose-bisphosphate aldolase, a microorganism expressing the tagatose-bisphosphate aldolase, or a culture of the microorganism, wherein the method may further comprise converting tagatose-6-phosphate into tagatose by contacting tagatose-6-phosphate with tagatose-6-phosphate phosphatase, a microorganism expressing the tagatose-6-phosphate phosphatase, or a culture of the microorganism.

Other objects and advantages of the present disclosure will be described in more detail with reference to the following description along with the accompanying claims and drawings. Since contents that are not described in the present specification may be sufficiently recognized and inferred by those skilled in the art or similar art, a description thereof will be omitted.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. **1**A to **1**D are results of HPLC chromatography showing that tagatose-bisphosphate aldolases (CJ_KO_F6P4E, CJ_RM_F6P4E, CJ_RP_6P4E, and CJ_LP_F6P4E) of one embodiment of the present disclosure have fructose-6-phosphate-4-epimerase activity;

FIGS. **2**A and **2**B are results of HPLC chromatography showing that treatment of fructose-6-phosphate with tagatose-bisphosphate aldolase (CJ_KO_F6P4E and CJ_RP_F6P4E) and tagatose-6-phosphate phosphatase (CJ_T4) converts fructose-6-phosphate into tagatose in one embodiment of the present disclosure;

FIG. **3** is a result of HPLC chromatography showing that T4 which is an enzyme of one embodiment of the present disclosure has tagatose-6-phosphate phosphatase activity;

FIG. **4** is a result of protein electrophoresis (SDS-PAGE) to analyze molecular weights of enzymes used in the production pathways of tagatose from starch, sucrose, or glucose in one embodiment of the present disclosure, wherein M represents a protein size ladder (size marker, Bio-FAD, USA);

FIG. **5** is a result of HPLC chromatography showing that TD1(CJ_TD1_F6P4E) which is an enzyme of one embodiment of the present disclosure has fructose-6-phosphate-4-epimerase activity; and

FIG. **6** is a result of HPLC chromatography showing that when all of the enzymes involved in the production pathway of tagatose from maltodextrin were added at the same time,

tagatose was produced by complex enzyme reactions, wherein TD1 (CJ_TD1_F6P4E) was used as tagatose-bisphosphate aldolase.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Hereinafter, the present disclosure will be described in detail as follows. Meanwhile, each description and embodiment disclosed in this disclosure may be applied to other descriptions and embodiments to common things. Further, all combinations of various elements disclosed in this disclosure fall within the scope of the present disclosure. Further, the scope of the present disclosure is not limited by the specific description described below.

To achieve one object of the present disclosure, an aspect of the present disclosure provides a composition for producing tagatose-6-phosphate, comprising tagatose-bisphosphate aldolase, a microorganism expressing the tagatose-bisphosphate aldolase, or a culture of the microorganism.

The tagatose-bisphosphate aldolase (EC 4.1.2.40) is known to produce glycerone phosphate and D-glyceraldehyde 3-diphosphate from D-tagatose 1,6-bisphosphate as a substrate, and to participate in a galactose metabolism. For example, the tagatose-bisphosphate aldolase may be any one without limitation as long as it is able to produce tagatose-6-phosphate from fructose-6-phosphate as a substrate.

Specifically, the tagatose-bisphosphate aldolase may be a polypeptide consisting of an amino acid sequence of SEQ ID NO: 1, 3, 5, 7, or 9, or comprise a polypeptide having at least 80%, 90%, 95%, 97%, or 99% homology with the amino acid sequence of SEQ ID NO: 1, 3, 5, 7, or 9. It is also apparent that a polypeptide having the homology and an amino acid sequence exhibiting the efficacy (i.e., fructose-6-phosphate C4-epimerization activity to convert fructose-6-phosphate into tagatose-6-phosphate by epimerizing fructose-6-phosphate at C4 position of fructose) corresponding to the protein consisting of the amino acid sequence of SEQ ID NO: 1, 3, 5, 7, or 9 is also included in the scope of the present disclosure, although it has an amino acid sequence, of which a partial sequence is deleted, modified, substituted, or added. Further, a probe which may be produced from the known nucleotide sequence, for example, a polypeptide encoded by a polynucleotide which is hybridizable with a complementary sequence to all or a part of a nucleotide sequence encoding the polypeptide under stringent conditions may be also included without limitation, as long as it has the fructose-6-phosphate C4-epimerization activity. Therefore, the composition for producing tagatose-6-phosphate may further comprise fructose-6-phosphate. Further, the composition may comprise one or more of tagatose-bisphosphate aldolase consisting of the amino acid sequence of 1, 3, 5, 7, or 9.

The present disclosure revealed that the 'tagatose-bisphosphate aldolase' exhibits the fructose-6-phosphate 4-epimerization activity to convert fructose-6-phosphate into tagatose-6-phosphate by epimerizing fructose-6-phosphate at C4 position. In the present disclosure, therefore, the 'tagatose-bisphosphate aldolase' may be used interchangeably with 'fructose-6-phosphate C4 epimerase'.

As used herein, the term "stringent conditions" means conditions under which specific hybridization between polynucleotides is allowed. These conditions depend on the length of the polynucleotide and the degree of complementation, and variables are well known in the art, and specifically described in a literature (e.g., J. Sambrook et al., infra). The stringent conditions may include, for example, conditions under which genes having high homology, 80% or higher homology, 90% or higher homology, 95% or higher homology, 97% or higher homology, or 99% or higher homology, are hybridized with each other and genes having homology lower than the above homology are not hybridized with each other, or ordinary washing conditions of Southern hybridization, i.e., washing once, specifically, twice or three times at a salt concentration and a temperature corresponding to 60° C., 1×SSC, 0.1% SDS, specifically, 60° C., 0.1×SSC, 0.1% SDS, and more specifically 68° C., 0.1×SSC, 0.1% SDS. The probe used in the hybridization may be a part of a complementary sequence of the nucleotide sequence. Such a probe may be produced by PCR using oligonucleotides produced based on the known sequence as primers and a DNA fragment containing these nucleotide sequences as a template. Further, those skilled in the art may adjust the temperature and the salt concentration of the washing solution according to factors such as the length of the probe, if necessary.

As used herein, the term "homology" refers to a percentage of identity between two polypeptide moieties. Sequence correspondence from one moiety to another may be determined by a known technique in the art. For example, the homology may be determined by directly aligning the sequence information of two polypeptide molecules, e.g., parameters such as score, identity, and similarity, etc., using a computer program that is readily available and capable of aligning sequence information (e.g., BLAST 2.0). Additionally, the homology between polynucleotides may be determined by hybridizing the polynucleotides under a condition for forming a stable double-strand in the homologous regions followed by digesting the hybridized strand by a single-strand-specific nuclease to determine the size of digested fragments.

In a specific embodiment, the fructose-6-phosphate-1-epimerase of the present disclosure may be an enzyme derived from a thermophilic microorganism or a variant thereof, for example, an enzyme derived from *Thermanaerothrix* sp. or a variant thereof, an enzyme derived from *Kosmotoga* sp. or a variant thereof, an enzyme derived from *Rhodothermus* sp. or a variant thereof, an enzyme derived from *Limnochorda* sp. or a variant thereof, and specifically, an enzyme derived from *Thermanaerothrix daxensis, Kosmotoga olearia, Rhodothermus marinus, Rhodothermus profundi*, or *Limnochorda pilosa*, but is not limited thereto.

The fructose-6-phosphate-4-epimerase of the present disclosure or a variant thereof is characterized by converting D-fructose-6-phosphate into D-tagatose-6-phosphate by epimerizing D-fructose-6-phosphate at C4 position. The fructose-t-phosphate-4-epimerase of the present disclosure may be an enzyme which is known to have tagatose-bisphosphate aldolase activity, and the tagatose-bisphosphate aldolase produces glycerine phosphate and D-glyceraldehyde 3-diphosphate from D-tagatose 1,6-bisphosphate as a substrate, and participates in a galactose metabolism. The present disclosure newly revealed that the tagatose-bisphosphate aldolase has the fructose-6-phosphate-4-epimerase activity. Accordingly, one embodiment of the present disclosure relates to novel use of the tagatose-bisphosphate aldolase including using the tagatose-bisphosphate aldolase as the fructose-6-phosphate-4-epimerase in the production of tagatose-6-phosphate from fructose-6-phosphate. Further, another embodiment of the present disclosure relates to a method of producing tagatose-6-phosphate from fructose-6-phosphate using the tagatose-bisphosphate aldolase as the fructose-6-phosphate-4-epimerase.

In one embodiment, the fructose-6-phosphate-4-epimerase of the present disclosure may be an enzyme having high heat resistance. Specifically, the fructose-6-phosphate-4-epimerase of the present disclosure may exhibit 50% to 100%, 60% to 100%, 70% to 100%, or 75% to 100% of its maximum activity at 50° C. to 70° C. More specifically, the fructose-6-phosphate-4-epimerase of the present disclosure may exhibit 80% to 100% or 85% to 100% of its maximum activity at 55° C. to 65° C., 60° C. to 70° C., 55° C., 60° C., or 70° C.

Furthermore, the fructose-6-phosphate-4-epimerase consisting of the amino acid sequence of SEQ ID NO: 1, 3, 5, 7, or 9 may be, hut is not limited to, encoded by a nucleotide sequence of SEQ ID NO: 2, 4, 6, 8, or 10, respectively.

The fructose-6-phosphate-4-epimerase of the present disclosure or a variant thereof may be obtained by transforming a microorganism such as *Escherichia. coli* with DNA expressing the enzyme of the present disclosure or the variant thereof, e.g., SEQ ID NO: 2, 4, 6, 8, or 10, culturing the microorganism to obtain a culture, disrupting the culture, and then performing purification using a column, etc. The microorganism for transformation may include *Corynebacterium glutamicum, Aspergillus oryzae*, or *Bacillus subtilis*, in addition to *Escherichia coli*. In a specific embodiment, the transformed microorganism may be *Escherichia. coli* BL21 (DE3)/CJ_KO_F6P4E, *Escherichia. coli* BL21(DE3)/CJ_RM_F6P4E, *Escherichia. coli* BL21(DE3)/CJ_RP_F6P4E, *Escherichia. coli* BL21(DE3)/CJ_LP_F6P4E, or *Escherichia. coli* BL21(DE3)/pBT7-C-His-CJ_td1. These microorganisms were deposited at the Korean Culture Center of Microorganisms which is an International Depositary Authority under the provisions of the Budapest Treaty with Accession No. KCCM1.1999P (*Escherichia. coli* BL21 (DE3)/CJ_KO_F6P4E) (date of deposit: Mar. 24, 2017), KCCM12096P (*Escherichia. coli* BL21(DE3)/CJ_RM_F6P4E) (date of deposit: Aug. 11, 2017), KCCM12097P (*Escherichia. coli* BL21(DE3)/CJ_RP_F6P4E) (date of deposit: Aug. 11, 2017), KCCM12095P (*Escherichia. coli* BL21(DE3)/CJ_LP_F6P4E) (date of deposit: Aug. 11, 2017), and KCCM11995P (*Escherichia. coli* BL21(DE3)/pBT7-C-His-CJ_-td1) (date of deposit: Mar. 20, 2017), respectively.

The fructose-6-phosphate-4-epimerase used in the present disclosure may be provided by using a nucleic acid encoding the same.

As used herein, the term "nucleic acid" means that it encompasses DNA or RNA molecules, wherein nucleotides which are basic constituent units in the nucleic acid may include not only natural nucleotides but also analogues with modification of sugar or base (see: Scheit, Nucleotide Analogs, John Wiley, New York (1980); Uhlman and Peyman, Chemical Reviews, 90:543-584(1990)).

The nucleic acid of the present, disclosure may be a nucleic acid encoding the polypeptide consisting of the amino acid sequence of SEQ ID NO: 1, 3, 5, 7, or 9 of the present disclosure or a nucleic acid encoding a polypeptide having at least 80%, 90%, 95%, 97% or 99% homology with the fructose-6-phosphate-4-epimerase of the present disclosure and having the fructose-6-phosphate-4-epimerase activity. For example, the nucleic acid encoding the fructose-6-phosphate-4-epimerase consisting of the amino acid sequence of SEQ ID NO: 1 may be a nucleic acid having at least 80%, 90%, 95%, 97%, 99% or 100% homology with the nucleotide sequence of SEQ ID NO: 2. Further, the nucleic acid encoding the fructose-6-phosphate-4-epimerase consisting of the amino acid sequence of SEQ ID NO: 3, 5, 7, or 9 may be a nucleic acid having at least 80%, 90%, 95%, 97%, 99% or 100% homology with the nucleotide sequence of SEQ ID NO: 4, 6, 8, or 10 corresponding thereto, respectively. It is also apparent that the nucleic acid of the present disclosure may include a nucleic acid which is translated into the fructose-6-phosphate-4-epimerase of the present disclosure due to codon degeneracy or a nucleic acid which hybridizes with a nucleic acid consisting of a nucleotide sequence complementary to the nucleotide sequence of SEQ ID NO: 2, 4, 6, 8, or 10 under stringent conditions and encodes the polypeptide having the fructose-6-phosphate-4-epimerase activity of the present disclosure.

The microorganism expressing the fructose-6-phosphate-4-epimerase which may be used in the present disclosure may be a microorganism comprising a recombinant vector comprising the nucleic acid.

The vector may be operably linked to the nucleic acid of the present disclosure. As used herein, the term "operably linked" means that a nucleotide expression regulatory sequence and a nucleotide sequence encoding a targeted protein are operably linked to each other to perform the general functions, thereby affecting expression of the encoding nucleotide sequence. The operable linkage to the vector may be produced using a genetic recombination technology known in the art, and the site-specific DNA cleavage and linkage may be produced using restriction enzymes and ligases known in the art.

As used herein, the term "vector" refers to any mediator for cloning and/or transferring of bases into an organism, such as a host cell. The vector may be a replicon that is able to bring the replication of combined fragments in which different DNA fragments are combined. Herein, the term "replicon" refers to any genetic unit (e.g., plasmid, phage, cosmid, chromosome, virus) which functions as a self-unit of DNA replication in vivo, i.e., which is able to be replicated by self-regulation. As used herein, the term "vector" may comprise viral and non-viral mediators for introducing the bases into the organism, e.g., a host cell, in vitro, ex vivo, or in vivo, and may also comprise a minicircular DNA, a transposon such as Sleeping Beauty (Izsvak et al. J. MoI. Biol. 302:93-102 (2000)), or an artificial chromosome. Examples of the vector commonly used may include natural or recombinant plasmids, cosmids, viruses, and bacteriophages. For example, as a phage vector or cosmid vector, pWE15, M13, MBL3, MBL4, IXII, ASHII, APII, t10, t11, Charon4A, and Charon21A, etc., may be used; and as a plasmid vector, those based on pBR, pUC, pBluescriptII, pCEM, pTZ, pCL, and pET, etc., may be used. The vectors that may be used in the present disclosure are not particularly limited, but any known expression vector may be used. Further, the vector may be a recombinant vector characterized by further comprising various antibiotic resistance genes. As used herein, the term "antibiotic resistance gene" refers to a gene having resistance against an antibiotic, and a cell having this gene survives in an environment treated with the corresponding antibiotic. Therefore, the antibiotic resistance gene is used as a selectable marker during production of a large amount of plasmids in *Escherichia. coli*. The antibiotic resistance gene in the present disclosure is not a factor that greatly influences expression efficiency according to optimal combinations of vectors which is a key technology of the present disclosure, and thus an antibiotic resistance gene that is generally used as a selectable marker may be used without limitation. Specific examples may include a resistance gene against ampicilin, tetracyclin, kanamycin, chloroamphenicol, streptomycin, or neomycin, etc.

The microorganism expressing the fructose-6-phosphate-4-epimerase which may be used in the present disclosure may be obtained by a method of introducing the vector comprising the nucleic acid encoding the enzyme into a host cell, and a method of transforming the vector may be any method as long as it is able to introduce the nucleic acid into the cell. An appropriate standard technique known in the art may be selected and performed. Electroporation, calcium phosphate co-precipitation, retroviral infection, microinjection, a DEAE-dextran method, a cationic liposome method, and a heat shock method may be included, but is not limited thereto.

As long as the transformed gene may be expressed in the host cell, it may be inserted into the chromosome of the host cell, or it may exist extrachromosomally. Further, the gene comprises DNA and RNA as a polynucleotide encoding a polypeptide, and any form may be used without limitation, as long as it may be introduced into the host cell and expressed therein. For example, the gene may be introduced into the host cell in the form of an expression cassette, which is a polynucleotide construct comprising all elements required for its autonomous expression. Commonly, the expression cassette may comprise a promoter operably linked to the gene, transcriptional termination signals, ribosome binding sites, and translation termination signals. The expression cassette may be in the form of a self-replicable expression vector. In addition, the gene as it is or in the form of a polynucleotide construct may be introduced into the host cell and operably linked to sequences required for expression in the host cell.

The microorganism of the present disclosure may include either a prokaryotic microorganism or a eukaryotic microorganism, as long as it is a microorganism capable of producing the fructose-6-phosphate-4-epimerase of the present disclosure by comprising the nucleic acid of the present disclosure or the recombinant vector of the present disclosure. For example, the microorganism may include microorganism strains belonging to the genus *Escherichia*, the genus *Erwinia*, the genus *Serratia*, the genus *Providencia*, the genus *Corynebacterium*, and the genus *Brevibacterium*, and specifically, it may be *Escherichia. coli* or *Corynebacterium glutamicum*, but is not limited thereto. Specific examples of the microorganism may include *Escherichia. coli* BL21(DE3)/CJ_KO_F6P4E, *Escherichia. Coli* BL21(DE3)/CJ_RM_F6P4E, *Escherichia. coli* BL21(DE3)/CJ_RP_F6P4E, *Escherichia. coli* BL21(DE3)/CJ_LP_F6P4E, *Escherichia. coli* BL21(DE3)/pBT7-C-His-CJ_td1, etc.

The microorganism of the present disclosure may include any microorganism capable of expressing the fructose-6-phosphate-4-epimerase of the present disclosure or related enzymes according to various known methods, in addition to introduction of the nucleic acid or the vector.

The culture of the microorganism of the present disclosure may be produced by culturing, in a medium, the microorganism capable of expressing the tagatose-bisphosphate aldolase of the present disclosure or related enzymes.

As used herein, the term "culturing" means that the microorganism is allowed to grow under appropriately controlled environmental conditions. The culturing process of the present disclosure may be carried out according to an appropriate medium and culture conditions known in the art. The culturing process may be easily adjusted by those skilled in the art according to the strain to be selected. The step of culturing the microorganism may be, but is not particularly limited to, carried out by a batch process, a continuous process, or a fed batch process etc. With regard to the culture conditions, a proper pH (e.g., pH 5 to 9, specifically pH 7 to 9) may be adjusted using a basic compound (e.g., sodium hydroxide, potassium hydroxide, or ammonia) or an acidic compound (e.g., phosphoric acid or sulfuric acid), but is not particularly limited thereto. Additionally, an antifoaming agent such as fatty acid polyglycol ester may be added during the culturing process to prevent foam generation. Additionally, oxygen or an oxygen-containing gas may be injected into the culture in order to maintain an aerobic state of the culture; or nitrogen, hydrogen, or carbon dioxide gas may be injected without the injection of a gas in order to maintain an anaerobic or microaerobic state of the culture. The culture temperature may be maintained from 25° C. to 40° C., and specifically, from 30° C. to 37° C., but is not limited thereto. The culturing may be continued until the desired amount of useful materials is obtained, and specifically for about 0.5 hours to about 60 hours, but is not limited thereto. Furthermore, the culture medium to be used may comprise, as carbon sources, sugars and carbohydrates (e.g., glucose, sucrose, lactose, fructose, maltose, molasses, starch, and cellulose), oils and fats (e.g., soybean oil, sunflower oil, peanut oil, and coconut oil), fatty acids (e.g., palmitic acid, stearic acid, and linoleic acid), alcohols (e.g., glycerol and ethanol), and organic acids (e.g., acetic acid) etc. These substances may be used individually or in a mixture, but are not limited thereto. Nitrogen sources may include nitrogen-containing organic compounds (e.g., peptone, yeast extract, meat extract, malt extract, corn steep liquor, soybean meal, and urea) or inorganic compounds (e.g., ammonium sulfate, ammonium chloride, ammonium phosphate, ammonium carbonate, and ammonium nitrate) etc. These nitrogen sources may also be used individually or in a mixture, but are not limited thereto. Phosphorus sources may include potassium dihydrogen phosphate, dipotassium hydrogen phosphate, or the corresponding sodium-containing salts etc. These nitrogen sources may also be used individually or in a mixture, but are not limited thereto. The culture medium may comprise essential growth stimulators, such as metal salts (e.g., magnesium sulfate or iron sulfate), amino acids, and vitamins.

Another aspect of the present disclosure provides a composition for producing tagatose, comprising tagatose-bisphosphate aldolase, a microorganism expressing the tagatose-bisphosphate aldolase, or a culture of the microorganism; and tagatose-6-phosphate phosphatase, the microorganism expressing the tagatose-6-phosphate phosphatase, or a culture of the microorganism.

The description of the composition for producing tagatose-6-phosphate may be also applied to the composition for producing tagatose. The tagatose-6-phosphate phosphatase of the present disclosure may be any protein without limitation, as long as it has activity to convert tagatose-6-phosphate into tagatose by eliminating a phosphate group of the tagatose-6-phosphate. The tagatose-6-phosphate phosphatase of the present disclosure may be an enzyme derived from a heat-resistant microorganism, for example, an enzyme derived from *Thermotoga* sp. or a variant thereof, specifically, an enzyme derived from *Thermotoga maritima* or a variant thereof.

According to one embodiment of the present disclosure, the tagatose-6-phosphate phosphatase of the present disclosure may be a protein which consists of an amino acid sequence of SEQ ID NO: 11, a sequence having a genetic homology of 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99% or 100% thereto, or a genetic homology within the range determined by any two values of the above values. Accord-

ing to one embodiment of the present disclosure, the tagatose-6-phosphate phosphatase consisting of the amino acid sequence of SEQ ID NO: 11 of the present disclosure may be encoded by a nucleotide sequence of SEQ ID NO: 12.

The composition for producing tagatose of the present disclosure may further comprise glucose-6-phosphate isomerase, a microorganism expressing the glucose-b-phosphate isomerase, or a culture of the microorganism. In the presence of the enzyme, glucose-6-phosphate may be isomerized to produce fructose-6-phosphate. The glucose-6-phosphate-isomerase of the present disclosure may include any protein without limitation, as long as it has activity to isomerize glucose-6-phosphate into fructose-6-phosphate. The glucose-6-phosphate-isomerase of the present disclosure may be an enzyme derived from a heat-resistant microorganism, for example, an enzyme derived from *Thermotoga* sp. or a variant thereof, specifically, an enzyme derived from *Thermotoga maritima* or a variant thereof. According to one embodiment of the present disclosure, the glucose-6-phosphate-isomerase of the present disclosure may be a protein which consists of an amino acid sequence of SEQ ID NO: 13, a sequence having a genetic homology of 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% thereto, or a homology within the range determined by any two values of the above values. According to one embodiment of the present disclosure, the glucose-6-phosphate-isomerase consisting of the amino acid sequence of SEQ ID NO: 13 of the present disclosure may be encoded by a nucleotide sequence of SEQ ID NO: 14.

The composition for producing tagatose of the present disclosure may further comprise phosphoglucomutase, a microorganism expressing the phosphoglucomutase, or a culture of the microorganism. The enzyme catalyzes a reversible reaction of converting glucose-1-phosphate into glucose-6-phosphate or converting glucose-6-phosphate into glucose-1-phosphate. The phosphoglucomutase of the present disclosure may include any protein without limitation, as long as it has activity to convert glucose-1-phosphate into glucose-6-phosphate or to convert glucose-6-phosphate into glucose-1-phosphate. The phosphoglucomutase of the present disclosure may be an enzyme derived from a boat-resistant microorganism, for example, an enzyme derived from *Thermotoga* sp. or a variant thereof, specifically, an enzyme derived from *Thermotoga neapolitana* or a variant thereof. According to one embodiment of the present disclosure, the phosphoglucomutase of the present disclosure may be a protein which consists of an amino acid sequence of SEQ ID NO: 15, a sequence having a genetic homology of 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% thereto, or within the range determined by any two values of the above values. According to one embodiment of the present disclosure, the phosphoglucomutase consisting of the amino acid sequence of SEQ ID NO: 15 of the present disclosure may be encoded by a nucleotide sequence of SEQ ID NO: 16.

The composition for producing tagatose of the present disclosure may further comprise glucokinase, a microorganism expressing the glucokinase, or a culture of the microorganism. The glucokinase of the present disclosure may include any protein without limitation, as long as it has activity to phosphorylate glucose. The glucokinase of the present disclosure may be an enzyme derived from a heat-resistant microorganism, for example, an enzyme derived from *Deinococcus* sp. or *Anaerolinea* sp., or a variant thereof, specifically, an enzyme derived from *Deinococcus geothermalis* or *Anaerolinea thermophila*, or a variant thereof. The glucokinase of the present disclosure may include any protein without limitation, as long as it has activity to convert glucose into glucose-6-phosphate. Specifically, the glucokinase of the present disclosure may be a phosphate-dependent glucokinase. According to one embodiment of the present disclosure, the glucokinase of the present disclosure may be a protein which consists of an amino acid sequence SEQ ID NO: 17 or 19, a sequence having a genetic homology of 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% thereto, or a genetic homology within the range determined by any two values of the above values. According to one embodiment of the present disclosure, the glucokinase consisting of the amino acid sequence of SEQ ID NO: 17 of the present disclosure may be encoded by a nucleotide sequence of SEQ ID NO: 18, and the glucokinase consisting of the amino acid sequence of SEQ ID NO: 19 of the present disclosure may be encoded by a nucleotide sequence of SEQ ID NO: 20.

The composition for producing tagatose of the present disclosure may further comprise α-glucan phosphorylase, starch phosphorylase, maltodextrin phosphorylase, or sucrose phosphorylase, a microorganism expressing the same, or a culture of the microorganism. The phosphorylase may include any protein without limitation, as long as it has activity to convert starch, maltodextrin, or sucrose into glucose-1-phosphate. The phosphorylase may be an enzyme derived from a heat-resistant microorganism, for example, an enzyme derived from *Thermotoga* sp. or a variant thereof, specifically, an enzyme derived from *Thermotoga neapolitana* or a variant thereof. The phosphorylase of the present disclosure may be a protein which consists of an amino acid sequence of SEQ ID NO: 21, a sequence having a genetic homology of 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% thereto, or a genetic homology within the range determined by any two values of the above values. According to one embodiment of the present disclosure, the phosphorylase consisting of the amino acid sequence of SEQ ID NO: 21 of the present disclosure may be encoded by a nucleotide sequence of SEQ ID NO: 22.

The composition for producing tagatose of the present disclosure may further comprise n-amylase, pullulanase, glucoamylase, sucrase, or isoamylase; a microorganism expressing the amylase, pullulanase, glucoamylase, sucrase, or isoamylase; or a culture of the microorganism expressing the amylase, pullulanase, glucoamylase, sucrase, or isoamylase.

The composition for producing tagatose of the present disclosure may comprise two or more enzymes of the above-described enzymes which may be used in the production of tagatose or transformants thereof individually, or a transformant transformed with nucleotides encoding the two or more enzymes.

The composition for producing tagatose of the present disclosure may further comprise 4-α-glucanotransferase, a microorganism expressing the 4-α-glucanotransferase, or a culture of the microorganism expressing the 4-α-glucanotransferase. The 4-α-glucanotransferase of the present disclosure may include any protein without limitation, as long as it has activity to convert glucose into starch, maltodextrin, or sucrose. The 4-α-glucanotransferase of the present disclosure may be an enzyme derived from a heat-resistant microorganism, for example, an enzyme derived from *Thermotoga* sp. or a variant thereof, specifically, an enzyme derived from *Thermotoga maritima* or a variant thereof. According to one embodiment of the present disclosure, the 4-α-glucanotransferase of the present disclosure may be a protein which consists of an amino acid sequence of SEQ ID NO: 23, a sequence having a genetic homology of 70%,

75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% thereto, or a genetic homology within the range determined by any two values of the above values. According to one embodiment of the present disclosure, the 4-α-glucanotransferase consisting of the amino acid sequence of SEQ ID NO: 23 of the present disclosure may be encoded by a nucleotide sequence of SEQ ID NO: 24.

Examples of the microorganisms which may be used in the above-described embodiments may include *Escherichia. coli* BL21(DE3)/pET21a-CJ_ct1, *Escherichia. coli* BL21 (DE3)/pET21a-CJ_ct2, *Escherichia. coli* BL21(DE3)/pET21a-CJ_tn1, *Escherichia. coli* BL21(DE3)/pET21a-CJ_tn2, and *Escherichia. coli* BL21(DE3)/pET21a-CJ_t4, etc. The recombinant microorganisms were deposited at Korean Culture Center of Microorganisms on Mar. 20, 2017 with Accession Nos. KCCM11990P (*Escherichia. coli* BL21 (DE3)/pET21a-CJ_ct1), KCCM11991P (*Escherichia. coli* BL21(DE3)/pET21a-CJ_ct2), KCCM11992P (*Escherichia. coli* BL21(DE3)/pET21a-CJ_tn1), KCCM11993P (*Escherichia. coli* BL21(DE3)/pET21a-CJ_tn2), KCCM11994P (*Escherichia. coli* BL21(DE3)/pET21a-CJ_t4), respectively.

The composition for producing tagatose of the present disclosure may further comprise a substance, a component, or a composition corresponding to a substrate of each of the above-described enzymes.

The composition for producing tagatose of the present disclosure may further comprise any suitable excipient commonly used in the corresponding composition for producing tagatose. The excipient may include, for example, a preservative, a wetting agent, a dispersing agent, a suspending agent, a buffer, a stabilizing agent, or an isotonic agent, etc., but is not limited thereto.

The composition for producing tagatose of the present disclosure may further comprise a metal. In one embodiment, the metal of the present disclosure may be a metal containing a divalent cation. Specifically, the metal of the present disclosure may be nickel, cobalt, aluminum, magnesium (Mg), or manganese (Mn). More specifically, the metal of the present disclosure may be a metal ion or a metal salt, and much more specifically, the metal salt may be $NiSO_4$, $MgSO_4$, $MgCl_2$, $NiCl_2$, $CoCl_2$, $COSO_4$, $MnCl_2$, or $MnSO_4$.

Another aspect of the present disclosure relates to a method of producing tagatose-6-phosphate, comprising converting fructose-6-phosphate into tagatose-6-phosphate by contacting fructose-6-phosphate with tagatose-bisphosphate aldolase, the microorganism expressing the tagatose-bisphosphate aldolase, or the culture of the microorganism.

The description of the composition for producing tagatose-6-phosphate may be also applied to the composition for producing tagatose.

Another aspect of the present disclosure relates to a method of producing tagatose, comprising converting fructose-6-phosphate into tagatose-6-phosphate by contacting fructose-6-phosphate with tagatose-bisphosphate aldolase, the microorganism expressing the tagatose-bisphosphate aldolase, or the culture of the microorganism. The method of producing tagatose may further comprise converting tagatose-6-phosphate into tagatose by contacting tagatose-6-phosphate with tagatose-6-phosphate phosphatase, the microorganism expressing the tagatose-6-phosphate phosphatase, or the culture of the microorganism.

The method of the present disclosure may further comprise converting glucose-6-phosphate into fructose-6-phosphate by contacting glucose-6-phosphate with the glucose-6-phosphate-isomerase of the present disclosure, the microorganism expressing the glucose-6-phosphate-isomerase, or the culture of the microorganism expressing the glucose-6-phosphate-isomerase.

The method of the present disclosure may further comprise converting glucose-1-phosphate into glucose-6-phosphate by contacting glucose-1-phosphate with the phosphoglucomutase of the present disclosure, the microorganism expressing the phosphoglucomutase, or the culture of the microorganism expressing the phosphoglucomutase.

The method of the present disclosure may further comprise converting glucose into glucose-6-phosphate by contacting glucose with the glucokinase of the present disclosure, the microorganism expressing the glucokinase, or the culture of the microorganism expressing the glucokinase.

The method of the present disclosure may further comprise converting starch, maltodextrin, or sucrose into glucose-1-phosphate by contacting starch, maltodextrin, sucrose, or a combination thereof with the α-glucan phosphorylase, starch phosphorylase, maltodextrin phosphorylase, or sucrose phosphorylase of the present disclosure, the microorganism expressing the phosphorylase, or the culture of the microorganism expressing the phosphorylase.

The method of the present disclosure may further comprise converting starch, maltodextrin, or sucrose into glucose by contacting starch, maltodextrin, sucrose, or a combination thereof with the α-amylase, pullulanase, glucoamylase, sucrase, or isoamylase; the microorganism expressing the α-amylase, pullulanase, glucoamylase, sucrase, or isoamylase; or the culture of the microorganism expressing the α-amylase, pullulanase, glucoamylase, sucrase, or isoamylase.

The method of the present disclosure may further comprise converting glucose into starch, maltodextrin, or sucrose by contacting glucose with the 4-α-glucanotransferase of the present disclosure, the microorganism expressing the 4-α-glucanotransferase, or the culture of the microorganism expressing the 4-α-glucanotransferase.

Each contacting in the method of the present disclosure may be performed under conditions of pH 5.0 to pH 9.0, 30° C. to 80° C., and/or for 0.5 hours to 48 hours. Specifically, the contacting of the present disclosure may be performed under a condition of pH 6.0 to pH 9.0 or pH 7.0 to pH 9.0. Further, the contacting of the present disclosure may be performed under a temperature condition of 35° C. to 80° C., 40° C. to 80° C., 45° C. to 80° C., 50° C., to 80° C., 55° C. to 80%, 60° C. to 80° C., 30° C. to 70° C., 35° C. to 70° C., 40° C. to 70° C., 45° C. to 70° C., 50° C. to 70° C., 55° C. to 70° C., 60° C., to 70° C., 30° C. to 65° C., 35° C. to 65° C., 40° C. to 65° C., 45° C. to 65° C., 50° C. to 65° C., 55° C. to 65° C., 30° C. to 60° C., 35° C. to 60° C., 40° C. to 60° C., 45° C. to 60° C., 50° C. to 60° C. or 55° C. to 60° C. Furthermore, the contacting of the present disclosure may be performed for 0.5 hours to 36 hours, 0.5 hours to 24 hours, 0.5 hours to 12 hours, 0.5 hours to 6 hours, 1 hour to 36 hours, 1 hour to 24 hours, 1 hour to 12 hours, 1 hour to 6 hours, 3 hours to 36 hours, 3 hours to 24 hours, 3 hours to 12 hours, 3 hours to 6 hours, 12 hours to 36 hours, or 18 hours to 30 hours.

In one embodiment, the contacting of the present disclosure may be performed in the presence of a metal, a metal ion, or a metal salt.

Another aspect of the present disclosure relates to a method of producing tagatose, comprising contacting the composition for producing tagatose described herein with starch, maltodextrin, sucrose, or a combination thereof; and phosphate.

In a specific embodiment of the present disclosure, a method of producing tagatose is provided, comprising:

converting glucose into glucose-6-phosphate by contacting glucose with the glucokinase of the present disclosure, the microorganism expressing the glucokinase, or the culture of the microorganism,

converting glucose-6-phosphate into fructose-6-phosphate by contacting glucose-6-phosphate with the glucose-6-phosphate-isomerase of the present disclosure, the microorganism expressing the glucose-6-phosphate-isomerase, or the culture of the microorganism,

converting fructose-6-phosphate into tagatose-6-phosphate by contacting fructose-6-phosphate with the fructose-6-phosphate-4-epimerase of the present disclosure, the microorganism expressing the fructose-6-phosphate-4-epimerase, or the culture of the microorganism, and

converting tagatose-6-phosphate into tagatose by contacting tagatose-6-phosphate with the tagatose-6-phosphate phosphatase of the present disclosure, the microorganism expressing the tagatose-6-phosphate phosphatase, or the culture of the microorganism.

The conversion reactions may be performed sequentially or in situ in the same reaction system. In the method, phosphate released from tagatose-6-phosphate by phosphatase may be used as a substrate of the glucokinase to produce glucose-6-phosphate. Therefore, phosphate is not accumulated, and as a result, a high conversion rate may be obtained.

In the method, glucose may be, for example, produced by converting starch, maltodextrin, or sucrose into glucose by contacting starch, maltodextrin, sucrose, or a combination thereof with α-glucan phosphorylase, starch phosphorylase, maltodextrin phosphorylase, sucrose phosphorylase of the present disclosure, the microorganism expressing the phosphorylase, or the culture of the microorganism expressing the phosphorylase. Therefore, the method according to a specific embodiment may further comprise converting starch, maltodextrin, or sucrose into glucose.

In another specific embodiment of the present disclosure, a method of producing tagatose is provided, comprising:

converting glucose-1-phosphate into glucose-6-phosphate by contacting glucose-1-phosphate with the phosphoglucomutase of the present disclosure, the microorganism expressing the phosphoglucomutase, or the culture of the microorganism,

converting glucose-6-phosphate into fructose-6-phosphate by contacting glucose-6-phosphate with the glucose-6-phosphate-isomerase of the present disclosure, the microorganism expressing the glucose-6-phosphate-isomerase, or the culture of the microorganism,

converting fructose-6-phosphate into tagatose-6-phosphate by contacting fructose-6-phosphate with the fructose-6-phosphate-4-epimerase of the present disclosure, the microorganism expressing the fructose-6-phosphate-4-epimerase, or the culture of the microorganism, and converting tagatose-6-phosphate into tagatose by contacting tagatose-6-phosphate with the tagatose-6-phosphate phosphatase of the present disclosure, the microorganism expressing the tagatose-6-phosphate phosphatase, or the culture of the microorganism.

The conversion reactions may be performed sequentially or in situ in the same reaction system.

In the method, glucose-1-phosphate may be, for example, produced by converting starch, maltodextrin, or sucrose into glucose-1-phosphate by contacting starch, maltodextrin, sucrose, or a combination thereof with α-glucan phosphorylase, starch phosphorylase, maltodextrin phosphorylase, sucrose phosphorylase of the present disclosure, the microorganism expressing the phosphorylase, or the culture of the microorganism expressing the phosphorylase. Therefore, the method according to a specific embodiment may further comprise converting starch, maltodextrin, or sucrose into glucose-1-phosphate. In this regard, phosphate released from tagatose-6-phosphate by phosphatase may be used as a substrate of the phosphorylase to produce glucose-1-phosphate. Therefore, phosphate is not accumulated, and as a result, a high conversion rate may be obtained.

The method may further comprise purifying the produced tagatose. The purification in the method is not particularly limited, and a method commonly used in the art to which the present disclosure pertains may be used. Non-limiting examples may include chromatography, fractional crystallization, and ion purification, etc. The purification method may be performed only by a single method or by two or more methods. For example, the tagatose product may be purified through chromatography, and separation of the sugar by the chromatography may be performed by utilizing a difference in a weak binding force between the sugar to be separated and a metal ion attached to an ion resin.

In addition, the present disclosure may further comprise performing decolorization, desalination, or both of decolorization and desalination before or after the purification step of the present disclosure. By performing the decolorization and/or desalination, it is possible to obtain a more purified tagatose product without impurities.

Hereinafter, the present disclosure will be described in more detail with reference to Examples. However, these Examples are provided for better understanding, and the disclosure is not intended to be limited by these Examples.

Example 1: Production of Recombinant Expression Vector and Transformant of Each Enzyme

To provide α-glucan phosphorylase, phosphoglucomutase, glucose-6-phosphate-isomerase, 4-α-glucanotransferase which are heat-resistant enzymes needed in the production pathway of tagatose of the present disclosure, nucleotide sequences expected as the enzymes (the above enzymes are represented by SEQ ID NO: 22(CT1), SEQ ID NO: 16(CT2), SEQ ID NO: 14(TN1), and SEQ ID NO: 24(TN2), respectively) were selected from a nucleotide sequence of a thermophilic microorganism, *Thermotoga neapolitana* or *Thermotoga maritima*, which is registered in Genbank.

Based on the selected nucleotide sequences, forward primers (SEQ ID NO: 21: CT1-Fp, SEQ ID NO: 27: CT2-Fp, SEQ ID NO: 29: TN1-Fp, SEQ ID NO: 31: TN2-Fp) and reverse primers (SEQ ID NO: 26: CT1-Rp, SEQ ID NO: 28: CT2-Rp, SEQ ID NO: 30: TN1-Rp, SEQ ID NO: 32: TN2-Rp) were designed and synthesized, and the gene of each enzyme was amplified by PCR using the above primers and a genomic DNA of the *Thermotoga neapolitana* as a template. Each amplified gene of the enzymes was inserted into pET21a (Novagen) which is a plasmid vector for expression in *Escherichia. coli* using restriction enzymes, NdeI and XhoI or SalI, thereby producing recombinant expression vectors designated as pET21a-CJ_ct1, pET21a-CJ_ct2, pET21a-CJ_tn1, pET21a-CJ_tn2, respectively.

Each of the expression vectors was transformed into *Escherichia. coli* BL21(DE3) according to a common transformation method [see Sambrook et al. 1989], thereby producing transformants (transformed microorganisms) designated as *Escherichia. coli* BL21(DE3)/pET21a-CJ_ct1, *Escherichia. coli* BL21(DE3)/pET21a-CJ_ct2, *Escherichia. coli* BL21(DE3)/pET21a-CJ_tn1, *Escherichia. coli* BL21

(DE3)/pET21a-CJ_tn2, respectively. These transformants were deposited at the Korean Culture Center of Microorganisms under the provisions of the Budapest Treaty on Mar. 20, 2017 with Accession Nos. KCCM11990P (*Escherichia. coli* BL21(DE3)/pET21a-CJ_ct1), KCCM11991P (*Escherichia. coli* BL21(DE3)/pET21a-CJ_ct2), KCCM11992P (*Escherichia. coli* BL21(DE3)/pET21a-CJ_tn1), and KCCM11993P (*Escherichia. coli* BL21(DE3)/pET21a-CJ_tn2), respectively.

Example 2: Production of Recombinant Enzymes

Escherichia. coli BL21(DE3)/pET21a-CJ_ct1, *Escherichia. coli* BL21(DE3)/pET21a-CJ_ct2, *Escherichia. coli* BL21(DE3)/pET21a-CJ_tn1, *Escherichia. coli* BL21(DE3)/pET21a-CJ_tn2 expressing each of the enzymes produced in Example 1 were seeded in a culture tube containing 5 ml of LB liquid medium, and then seed culture was performed in a shaking incubator at 37° C. until absorbance at 600 nm reached 2.0.

Each of the cultures obtained by the seed culture was seeded in a culture flask containing an LB liquid medium, and then main culture was performed. When absorbance at 600 nm reached 2.0, 1 mM IPTG was added to induce expression and production of the recombinant enzymes. During the culture, a shaking speed was maintained at 180 rpm and a culture temperature was maintained at 37° C. Each culture was centrifuged at 8,000×g and 4° C. for 20 minutes to recover cells. The recovered cells were washed with 50 mM Tris-HCl (pH 8.0) buffer twice and suspended in the same buffer, followed by cell disruption using a sonicator. Cell lysates were centrifuged at: 13,000×g and 4° C. for 20 minutes to obtain only supernatants. Each enzyme was purified therefrom using His-tag affinity chromatography. The purified recombinant enzyme solution was dialyzed against 50 mM Tris-HCl (pH 8.0) buffer, and used for reaction.

A molecular weight of each purified enzyme was examined by SDS-PAGE, and as a result, it was confirmed that CT1 (α-glucan phosphorylase) has a molecular weight of about 96 kDa, CT2 (phosphoglucomutase) has a molecular weight of about 53 kDa, TN1 (glucose-6-phosphate-isomerase) has a molecular weight of about 51 kDa.

Example 3: Examination of Fructose-6-Phosphate-4-Epimerase Activity of Tagatose-Bisphosphate Aldolase

3-1. Production of Recombinant Expression Vector and Recombinant Microorganism Comprising Tagatose-Bisphosphate Aldolase Gene

To identify a novel heat-resistant fructose-6-phosphate-4-epimerase, genetic information of tagatose-bisphosphate aldolase derived from *Kosmotoga olearia, Rhodothermus marinus, Rhodothermus profundi*, and *Limnochorda pilosa* which are thermophilic microorganisms was acquired to produce recombinant vectors expressible in *Escherichia. coli* and recombinant microorganisms.

In detail, a nucleotide sequence of tagatose-bisphosphate aldolase was selected from nucleotide sequences of *Kosmotoga olearia* or *Rhodothermus marinus* ATCC 43812, *Rhodothermus profundi* DSM 22212, and *Limnochorda pilosa* DSM 28787, which are registered in Genbank and KEGG (Kyoto Encyclopedia of Genes and Genomes), and based on information of amino acid sequences (SEQ ID NOS: 1, 3, 5 and 7) and nucleotide sequences (SEQ ID NOS: 2, 4, 6 and 8) of the four microorganisms, pBT7-C-His-CJ_KO_F6P4E, pBT7-C-His-CJ_RM_F6P4E, pBT7-C-His-CJ_RP_F6P4E, and pBT7-C-His-CJ_LP_F6P4E which are recombinant vectors comprising the nucleotide sequence of the enzyme and being expressible in *Escherichia. coli* were produced (Bioneer Corp., Korea).

Each of the produced expression vectors was transformed into *Escherichia. coli* BL21(CE3) by heat shock transformation (Sambrook and Russell: Molecular cloning, 2001) to produce recombinant microorganisms, and used after being frozen and stored in 50% glycerol. The recombinant microorganisms were designated as *Escherichia. coli* BL21(DE3)/CJ_KO_F6P4E, *Escherichia. coli* BL21(DE3)/CJ_RM_F6P4E, *Escherichia. coli* *BL*21(DE3)/CJ_RP_F6P4E, and *Escherichia. coli* BL21(DE3)/CJ_LP_F6P4E, respectively and deposited at the Korean Culture Center of Microorganisms (KCCM) which is an International Depositary Authority under the provisions of the Budapest Treaty with Accession Nos. KCM11999P (date of deposit: Mar. 24, 2017), KCCM12096P (dace of deposit: Aug. 11, 2017), KCCM12097P (date of deposit: Aug. 11, 2017), and KCCM12095P (date of deposit: Aug. 11, 2017), respectively.

To identify an additional novel heat-resistant fructose-6-phosphate-4-epimerase, a nucleotide sequence expected as the enzyme was selected from a nucleotide sequence of a thermophilic *Thermanaerothrix daxensis*, which is registered in Genbank, and based on information of an amino acid sequence (SEQ ID NO: 9) and a nucleotide sequence (SEQ ID NO: 10) of the microorganism, the gene was inserted into pBT7-C-His (Bioneer Corp.,) which is a recombinant vector comprising the nucleotide sequence of the enzyme and being expressible in *Escherichia. coli* to produce a recombinant expression vector designated as pBT7-C-His-CJ_td1. The expression vector was transformed into an *Escherichia. coli* βL21 (DE3) strain by a common transformation method [see Sambrook et al. 1989] to produce a transformant (transformed microorganism) designated as *Escherichia. coli* *BL*21(DE3)/pBT7-C-His-CJ_td1, and this transformant was deposited at the Korean Culture Center of Microorganisms (KCCM) under the provisions of the Budapest Treaty on Mar. 20, 2017 with Accession No. KCCM11995P (*Escherichia. coli* BL21 (DE3)/pBT7-C-His-CJ_td1).

3-2. Production of Recombinant Tagatose-Bisphosphate Aldolase Enzyme

To produce recombinant enzymes, CJ_KO_F6P4E, CJ_RM_F6P4E, CJ_RP_F6P4E, CJ_LP_F6P4E, and CJ_TD1_F6P4E from the produced recombinant microorganisms, each of the recombinant microorganisms was seeded in a culture tube containing 5 ml of an LB liquid medium with ampicillin antibiotic, and then seed culture was performed in a shaking incubator at 37° C. until absorbance at 600 nm reached 2.0. Each of the cultures obtained by the seed culture was seeded in a culture flask containing an LB liquid medium, and then main culture was performed. When absorbance at 600 nm reached 2.0, 1 mM IPTG (isopropyl β-D-1-thiogalactopyranoside) was added to induce expression and production of the recombinant enzyme. The seed culture and the main culture were performed under conditions of 180 rpm and 37° C. Each culture of the main culture was centrifuged at 8,000×g and 4° C. for 20 minutes to recover cells. The recovered cells were washed with 25 mM Tris-HCl (pH 7.0) buffer twice and suspended in the same buffer, followed by cell disruption using a sonicator. Each cell lysate was centrifuged at 13,000×g and 4° C. for 20 minutes to take only a supernatant. The supernatant was purified using His-tax affinity

chromatography, and 10 column volumes of 50 mM NaH_2PO_4 (pH 8.0) buffer containing 20 mM imidazole and 300 mM NaCl was applied to remove non-specifically bound proteins. Next, 50 mM Na_2HPO_4 (pH 8.0) buffer containing 250 mM imidazole and 300 mM NaCl was further applied to perform elution and purification. Dialysis was performed using 25 mM Tris-HCl (pH 7.0) buffer to obtain CJ_KO_F6P4E, CJ_RM_F6P4E, CJ_RP_F6P4E, CJ_LP_F6P4E, and CJ_TD1_F6P4E which are purified enzymes for analysis of enzyme characterization.

3-3. Analysis of Fructose-6-Phosphate-4-Epimerase Activity of Recombinant Tagatose-Bisphosphate Aldolase Enzyme

The fructose-6-phosphate-4-epimerase activities of the recombinant tagatose-bisphosphate aldolase enzymes obtained in Example 3-2 were analyzed. In detail, 1% by weight of fructose-6-phosphate as a substrate was suspended in 25 mM Tris-HCl (pH 7.0) buffer, and each 1 unit/mi of the purified CJ_KO_F6P4E, CJ_RM_6P4E, CJ_RP_F6P4E, CJ_LP_F6P4E, and CJ_TD1_F6P4E was added thereto, and allowed to react at 60° C. for 1 hour. To remove phosphate, 1 unit/ml of phosphatase (Alkaline phosphatase of NEB, Calf Intestinal) was added and allowed to react at 37° C. for 1 hour. Reaction products were analyzed by HPLC, and HPLC analysis was performed under conditions of using a SP0810 (Shodex) column and applying a mobile phase (water) at 80° C. and a flow rate of 1 mil/min, and resultants were analyzed using a refractive index detector.

As a result, it was confirmed that all of CJ_KO_F6P4E, CJ_RM_F6P4E, CJ_RP_F5P4E, and CJ_LP_F6P4E have the activity to convert fructose-6-phosphate into tagatose-6-phosphate (FIGS. **1**A to **1**D).

It was also confirmed that. CJ_TD1_F6P4E has activity to convert fructose-6-phosphate into tagatose-6-phosphate (FIG. **5**).

Example 4: Identification of tagatose-6-phosphate Phosphatase (D-tagatose-6-phosphate Phosphatase)

To perform production of tagatose from fructose-6-phosphate by simultaneous complex enzyme reactions in the tagatose production pathway of the present disclosure, tagatose-6-phosphate phosphatase which is able to exert the simultaneous enzyme reaction together with tagatose-bisphosphate aldolase was identified.

4-1. Production of Recombinant Expression Vector and Recombinant Microorganism Comprising Tagatose-6-Phosphate Phosphatase Gene

A nucleotide sequence (SEQ ID NO: 12, hereinafter, referred to as t4) and an amino acid sequence (SEQ ID NO: 11) expected as the tagatose-6-phosphate phosphatase were selected from a nucleotide sequence of *Thermotoga maritima*, which is registered in Genbank, and based on the selected nucleotide sequence, a forward primer (SEQ ID NO: 33) and a reverse primer (SEQ. ID NO: 34) were designed and synthesized. Polymerase chain reaction (PCR) was performed using the primers and genomic DNA of *Thermotoga maritima* as a template to amplify t4 gene. The amplified gene was inserted into pET21a (Novagen) which is a plasmid vector for expression in *Escherichia. coli* using restriction enzymes NdeI and XhoI, thereby producing a recombinant expression vector which was then designated as pET21a-CJ_t4. The produced expression vector was transformed into *Escherichia. coli* BL21(DE3) strain by heat shock transformation (Sambrook and Russell: Molecular cloning, 2001) to produce a recombinant microorganism, which was then used after being frozen and stored in 50% glycerol. The recombinant microorganism was designated as *Escherichia. coli* BL21(DE3)/pET21a-CJ_t4, and deposited at the Korean Culture Center of Microorganisms (KCCM) which is an International Depositary Authority under the provisions of the Budapest Treaty on Mar. 20, 2017 with Accession No. KCCM11994P.

4-2. Production of Recombinant Tagatose-6-Phosphate Phosphatase

Escherichia. coli BL21(DE3)/pET21a-CJ_t4 was seeded in a culture tube containing 5 ml of LB liquid medium and then seed culture was performed in a shaking incubator at 37° C. until absorbance at 600 nm reached 2.0. The culture obtained by the seed culture was seeded in a culture flask containing an L3 liquid medium, and then main culture was performed. When absorbance at 600 nm reached 2.0, 1 mM IPTG was added to induce expression and production of the recombinant enzymes. The seed culture and the main culture were performed at a shaking speed of 180 rpm and 37° C. The culture obtained by the main culture was centrifuged at 8,000×g and 4° C. for 20 minutes to recover cells. The recovered cells were washed with 50 mM Tris-HCl (pH 8.0) buffer twice and suspended in the same buffer, followed by cell disruption using a sonicator. A cell lysate was centrifuged at 13,000×g and 4° C. for 20 minutes to obtain only a supernatant. The enzyme was purified therefrom using His-tag affinity chromatography. The purified enzyme was used after dialysis against 50 mM Tris-HCl. (pH 8.0) buffer, and the purified recombinant enzyme was designated as CJ_T4.

4-3. Analysis of Tagatose-6-Phosphate Phosphatase Activity of CJ_T4

To analyze activity of CJ_T4, tagatose-6-phosphate was suspended in 50 mM Tris-HCl (pH 7.5) buffer, and 0.1 unit/ml of the purified CJ_T4 and 10 mM $MgCl_2$ were added thereto and allowed to react at 70° C. for 10 minutes. Then, the reaction product was analyzed by HPLC. HPLC analysis was performed under conditions of using a HPX-37H (Bio-Rad) column and applying a mobile phase (water) at 60° C. and a flow rate of 0.6 ml/min, and tagatose and tagatose-6-phosphate were analyzed using a refractive index detector.

As a result, tagatose was produced in the reaction product. As a result of performing the same reaction after adding CJ_T4 to phosphate and tagatose reactants, no tagatose was produced, indicating that CJ_T4 has irreversible tagatose-6-phosphate phosphatase activity (FIG. **3**).

Example 5: Production of Tagatose by Simultaneous Complex Enzyme Reactions

1% (w/v) fructose-6-phosphate suspended in 25 mM Tris-HCl (pH 7.0) buffer was added to a mixed enzyme solution of 1 unit/ml of CJ_KO_F6P4E or CJ_RP_F6P4E and 1 unit/ml of CJ_t4 (Accession No. KCCM11994P), and allowed to react at 60° C. for 1 hour, and then HPLC was performed to analyze the reaction product. HPLC analysis was performed under conditions of using a SP0810 (Shodex) column and applying a mobile phase (water) at 80° C. and a flow rate of 1 ml/min, and tagatose Was detected using a refractive index detector.

As a result, tagatose production was observed, indicating that tagatose may be produced from fructose-6-phosphate by simultaneous complex enzyme reactions of tagatose-bisphosphate aldolase and tagatose-6-phosphate phosphatase (FIGS. **2**A and **2**B).

Example 6: Production of Tagatose from Maltodextrin by Simultaneous Complex Enzyme Reactions

To analyze the activity to produce tagatose from maltodextrin by complex enzymes, 5% (w/v) maltodextrin was added to a reaction solution containing 1 unit/ml of CT1, 1 unit/ml of CT2, 1 unit/ml of TN1, 1 unit/ml of T4, 1 unit/ml of TD1, 20 mM to 50 mM of sodium phosphate (pH 7.0), and allowed to react at 60° C. for 1 hour, and then HPLC was performed to analyze the reaction product. HPLC analysis was performed under conditions of using a SP0810 (Shodex) column and applying a mobile phase (water) at 80° C. and a flow rate of 0.6 ml/min, and tagatose was detected using a refractive index detector.

As a result, it was confirmed that tagatose may be produced from maltodextrin by the complex enzyme reactions of added CT1, CT2, TN1, T4, and TD1 (FIG. **6**).

Effect of the Invention

A method of producing tagatose according to the present disclosure is economical because of using glucose or starch as a raw material, accumulates no phosphate to achieve a high conversion rate, and comprises a tagatose-6-phosphate phosphatase reaction which is an irreversible reaction pathway, thereby remarkably increasing a conversion rate into tagatose.

Further, tagatose may be produced from glucose or starch as a raw material by complex enzyme reactions, and thus there are advantages that the method is simple and economical, and a yield is improved.

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Leu Thr Lys Lys Ala Phe Phe Asp His Gly Leu Tyr Glu Ala Trp Gly
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Leu Lys Val Gly Pro Ala Leu Thr Phe Ala Leu Arg Glu Ala Phe Phe
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Ala Leu Ser Ser Ile Glu Lys Glu Leu Phe Tyr Asp Thr Pro Gly Leu
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Cys Ser Asn Phe Val Glu Val Val Glu Arg Ala Met Leu Asp Asn Pro
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Lys His Trp Glu Lys Tyr Tyr Gln Gly Glu Glu Arg Glu Asn Arg Leu
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Val Thr Arg Ala Ala Leu Glu Ala Ala Ala Glu Ala Asn Ala Pro Leu
    50                  55                  60

Leu Phe Ala Ala Thr Leu Asn Gln Val Asp Leu Asp Gly Gly Tyr Thr
65                  70                  75                  80

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Ile Arg Arg Ala Val Val Glu Ser Gly Arg Trp Thr Lys Trp Leu Gln
                325                 330                 335

Pro Glu Glu Lys Gly Gln Pro Phe Asp Ala Leu Asp Pro Glu Arg Gln
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    370                 375                 380

Asp Ala Asp Ala Phe Val Arg Thr Arg Ile Lys Ala Arg Leu Met Asp
385                 390                 395                 400

Tyr Phe Arg Ala Phe Asn Leu Ile His Phe Asn Glu Arg Leu Gln Ala
                405                 410                 415

Phe Leu Pro Glu
            420
```

<210> SEQ ID NO 4
<211> LENGTH: 1263
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Nucleic acid sequence of CJ_RM_F6P4E

<400> SEQUENCE: 4

```
atgcaggcgc aggccctgct gaccgttcca tttgatcggg tggcgaccca cgcacgcggg     60

tttgtgggct gggtggccga actgctgcag gggcccctgg cctatcagca tacgctgctg    120

gctgtctgtc ccaattcgga agcggtaaca cgggccgcgc tggaggccgc cgccgaggcc    180

aacgccccgc tgctttttgc cgccacgctg aaccaggtgg acctcgacgg cggctacacc    240

ggctggacgc ccgccacgct ggcccggttc gtggcggacg aactggcccg cctggacctg    300

cacatccccg tcgtgctcgg cctggaccac ggcggccccc ggaaaaagga tctgcacgcc    360

cgcaaccgat tgtcctttga ggaaaccttc caggccgtgc tgcgggccat cgaggcctgt    420

ctggatgccg gctacggcct gctgcacctg gatccgacgg tcgatctgga gctatcgccc    480

ggcacgccgg tgcccatccc gcgcattgtc gaacgctcgg tagcgctttt gcgtcatgcc    540

gaaacctatc gacttcgacg taacctgccg ccggtcgcct acgaggtggg caccgaagaa    600
```

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```
gtcggcggcg gcctgcaggc cgaagcgcgc atggcggagt ttctggatcg cctctggacc    660

gcactggacc gggaaggcct gccccatcca gtcttcgtgg tgggcgacat cggcacccgg    720

ctcgacacgc gcacgttcga cttcgagcgg gcccgacggc tggacgcgct ggtgcgccgc    780

tacggtgccc tcatcaaagg gcactacacc gacgacgtgg atcgcctcga tctgtacccg    840

aaggcgggca tcggcggggc caacgtgggc ccgggcctgg ccgccatcga gtttgaagcg    900

ctggaggcgc tggtggagga agcccgtcgc cgcggtcttt cggtgacgtt cgatcaggcc    960

atccgccggg ccgtcgtcga aagcggacgc tggacgaagt ggctccaacc ggaagagaaa   1020

ggccagccgt tcgatgcgct ggatcccgag cggcaacgct ggctggtggc caccggcagc   1080

cgctacgtgt ggacgcatcc ggccgtcctg caggcccgcc gcgaactcta cgaggcgctc   1140

gccccctggc tcgatgccga cgctttcgtg cgcacgcgca tcaaagcacg cctgatggac   1200

tactttcgtg ccttcaacct gatccatttc aacgagcggc tgcaggcctt tctccccgaa   1260

tga                                                                 1263
```

<210> SEQ ID NO 5
<211> LENGTH: 420
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amino acid sequences of CJ_RP_F6P4E

<400> SEQUENCE: 5

```
Met Gln Ala His Val Leu Leu Ala Pro Ser Phe Glu Gln Leu Ala Asp
1               5                   10                  15

His Arg His Gly Phe Val Gly Trp Leu Val Asp Leu Leu Arg Gly Pro
            20                  25                  30

Leu Ala Tyr Arg His Thr Leu Leu Ala Val Cys Pro Asn Ser Glu Ala
        35                  40                  45

Val Thr Arg Ala Ala Leu Glu Ala Ala Arg Glu Ala Asn Ala Pro Leu
    50                  55                  60

Phe Phe Ala Ala Thr Leu Asn Gln Val Asp Leu Asp Gly Gly Tyr Thr
65                  70                  75                  80

Gly Trp Thr Pro Ala Thr Leu Ala Arg Phe Val Ala Asp Glu Arg Ile
                85                  90                  95

Arg Leu Gly Leu Arg Ala Pro Val Val Leu Gly Leu Asp His Gly Gly
            100                 105                 110

Pro Trp Lys Lys Asp Trp His Val Arg Asn Arg Leu Pro Tyr Glu Ala
        115                 120                 125

Thr Leu Gln Ala Val Leu Arg Ala Ile Glu Ala Cys Leu Asp Ala Gly
    130                 135                 140

Tyr Gly Leu Leu His Leu Asp Pro Thr Val Asp Leu Glu Leu Pro Pro
145                 150                 155                 160

Gly Thr Pro Val Pro Ile Pro Arg Ile Val Glu Arg Thr Val Ala Leu
                165                 170                 175

Leu Gln His Ala Glu Thr Tyr Arg Gln Gln Arg Arg Leu Pro Pro Val
            180                 185                 190

Ala Tyr Glu Val Gly Thr Glu Glu Val Gly Gly Gly Leu Gln Ala Glu
        195                 200                 205

Ala Arg Met Ala Glu Phe Leu Asp Arg Leu Trp Thr Val Leu Asp Arg
    210                 215                 220

Glu Gly Leu Pro Arg Pro Val Phe Val Val Gly Asp Ile Gly Thr Arg
225                 230                 235                 240
```

```
Leu Asp Thr His Thr Phe Asp Phe Glu Arg Ala Arg Arg Leu Asp Ala
                245                 250                 255

Leu Val Arg Arg Tyr Gly Ala Leu Ile Lys Gly His Tyr Thr Asp Gly
            260                 265                 270

Val Asp Arg Leu Asp Leu Tyr Pro Gln Ala Gly Ile Gly Gly Ala Asn
        275                 280                 285

Val Gly Pro Gly Leu Ala Ala Ile Glu Phe Glu Ala Leu Glu Ala Leu
    290                 295                 300

Val Ala Glu Ala His Arg Arg Lys Leu Pro Val Thr Phe Asp Arg Thr
305                 310                 315                 320

Ile Arg Gln Ala Val Ile Glu Ser Gly Arg Trp Gln Lys Trp Leu Arg
                325                 330                 335

Pro Glu Glu Lys Gly Arg Pro Phe Glu Ala Leu Pro Pro Glu Arg Gln
            340                 345                 350

Arg Trp Leu Val Ala Thr Gly Ser Arg Tyr Val Trp Thr His Pro Ala
        355                 360                 365

Val Arg Gln Ala Arg His Gln Leu Tyr Gln Val Leu Ala Pro Trp Leu
    370                 375                 380

Asp Ala Asp Ala Phe Val Arg Ala Arg Ile Lys Ala Arg Leu Met Asp
385                 390                 395                 400

Tyr Phe Arg Ala Phe Asn Leu Ile Gly Phe Asn Glu Arg Leu Gln Ala
                405                 410                 415

Phe Leu Pro Asn
            420
```

<210> SEQ ID NO 6
<211> LENGTH: 1263
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Nucleic acid sequence of CJ_RP_F6P4E

<400> SEQUENCE: 6

```
atgcaggcgc acgtcctgct tgccccttcg ttcgagcagc tagcagacca caggcacgga     60

tttgttggct ggttggtcga tttgctgcgc ggaccgctgg cttaccggca cacgctgctg    120

gccgtatgtc ccaattccga agccgtaacg cgcgccgccc tggaagctgc gcgcgaagcc    180

aacgccccgc tatttttttgc ggctaccctg aaccaggtcg acctggatgg cggatatacc    240

ggctggaccc cggccacgct ggctcgtttt gttgccgacg agcgcatccg cctgggcctt    300

cgcgcccctg tcgtacttgg tctggatcac ggtggcccct ggaaaaagga ttggcatgtc    360

cgcaaccgtc ttccgtacga ggcaacgctc caggcggtgc ttcgcgcgat tgaggcctgc    420

ctcgacgcag gttatgggct gcttcatctg gacccgacgg tagatctgga attgccgccc    480

ggcacacccg tccccatccc acgtattgtc gaacgaacgg tagcgctttt acaacatgct    540

gaaacgtatc gccaacagcg tcgcctgccc ccggtcgcct acgaggtagg cacggaggag    600

gttggcggcg gcctgcaggc tgaggcgcga atggcagaat ttctggatcg actctggacc    660

gtcctggatc gggaagggct accccgtccg gtgtttgtgg tgggtgacat tggcacccgg    720

cttgacacgc acaccttcga ctttgaacgc gcccgtcgcc tggatgccct ggtgcgccgc    780

tacggtgccc tgatcaaggg gcactacacc gatggagtag accgcctgga tctatatcca    840

caggcgggta tcggtggagc aaacgtgggg cctggcctgg ctgctatcga gtttgaagcg    900

ctggaggccc tggtggccga agcgcaccgc cgcaagctgc ccgttacctt tgaccggacc    960
```

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atccgccagg ctgtcattga aagtggacgc tggcaaaaat ggctgcgccc tgaagagaaa   1020

ggacgtccct ttgaagcatt acctccagaa cgccagcggt ggctggtcgc tacaggcagc   1080

cgctacgtgt ggacgcaccc ggctgtccgg caggcgcgcc atcaattgta tcaggtgctc   1140

gctccctggc tcgatgccga tgcttttgtg cgcgcgcgca tcaaggcccg cctgatggac   1200

tacttccgcg ctttcaacct gataggcttc aatgaacggc tgcaggcctt tttacctaat   1260

tga                                                                 1263


<210> SEQ ID NO 7
<211> LENGTH: 448
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amino acid sequences of CJ_LP_F6P4E

<400> SEQUENCE: 7

Met Gln Thr Ser Thr Ala Tyr Val Arg Gln Val Ile Trp Gly Gln Gly
1               5                   10                  15

Thr Arg Asp Pro Arg Gly Ile Tyr Ser Val Cys Thr Ala Asp Pro Leu
            20                  25                  30

Val Leu Arg Ala Ala Leu Lys Gln Ala Val Glu Asp Gly Ser Pro Ala
        35                  40                  45

Leu Ile Glu Ala Thr Ser Asn Gln Val Asn Gln Phe Gly Gly Tyr Thr
    50                  55                  60

Gly Met Glu Pro Pro Ala Phe Val Glu Phe Val Leu Gly Leu Ala Arg
65                  70                  75                  80

Glu Met Gly Leu Pro Pro Glu Arg Leu Ile Leu Gly Gly Asp His Leu
                85                  90                  95

Gly Pro Asn Pro Trp Gln Arg Leu Ala Ala Glu Glu Ala Met Arg His
            100                 105                 110

Ala Cys Asp Leu Val Glu Ala Phe Val Ala Cys Gly Phe Thr Lys Ile
        115                 120                 125

His Leu Asp Ala Ser Met Pro Leu Gly Glu Glu Arg Ala Gly Gly Ala
    130                 135                 140

Leu Ser Lys Arg Val Val Ala Glu Arg Thr Ala Gln Leu Cys Glu Ala
145                 150                 155                 160

Ala Glu Ala Ala Phe Arg Lys Arg Ser Gln Ala Glu Gly Ala Ser Ala
                165                 170                 175

Pro Pro Leu Tyr Val Ile Gly Ser Asp Val Pro Pro Pro Gly Gly Glu
            180                 185                 190

Thr Ser Gly Ser Gln Gly Pro Lys Val Thr Thr Pro Glu Glu Phe Glu
        195                 200                 205

Glu Thr Val Ala Leu Thr Arg Ala Thr Phe His Asp Arg Gly Leu Asp
    210                 215                 220

Asp Ala Trp Gly Arg Val Ile Ala Val Val Val Gln Pro Gly Val Asp
225                 230                 235                 240

Phe Gly Glu Trp Gln Val His Pro Tyr Asp Arg Ala Ala Ala Ala Ser
                245                 250                 255

Leu Thr Arg Ala Leu Thr Gln His Pro Gly Leu Ala Phe Glu Gly His
            260                 265                 270

Ser Thr Asp Tyr Gln Thr Pro Gly Arg Leu Arg Gln Met Ala Glu Asp
        275                 280                 285

Gly Ile Ala Ile Leu Lys Val Gly Pro Ala Leu Thr Phe Ala Lys Arg
    290                 295                 300
```

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```
Glu Ala Leu Phe Ala Leu Asn Ala Leu Glu Ser Glu Val Leu Gly Thr
305                 310                 315                 320

Asp Gly Arg Ala Arg Arg Ser Asn Val Glu Ala Ala Leu Glu Glu Ala
                325                 330                 335

Met Leu Ala Asp Pro Arg His Trp Ser Ala Tyr Tyr Ser Gly Asp Glu
            340                 345                 350

His Glu Leu Arg Leu Lys Arg Lys Tyr Gly Leu Ser Asp Arg Cys Arg
        355                 360                 365

Tyr Tyr Trp Pro Val Pro Ser Val Gln Glu Ala Val Gln Arg Leu Leu
    370                 375                 380

Gly Asn Leu Arg Glu Ala Gly Ile Pro Leu Pro Leu Leu Ser Gln Phe
385                 390                 395                 400

Leu Pro Arg Gln Tyr Glu Arg Val Arg Glu Gly Val Leu Arg Asn Asp
                405                 410                 415

Pro Glu Glu Leu Val Leu Asp Arg Ile Arg Asp Val Leu Arg Gly Tyr
            420                 425                 430

Ala Ala Ala Val Gly Thr Gly Ala Arg Arg Ala Glu Pro Ser Pro Ala
        435                 440                 445


<210> SEQ ID NO 8
<211> LENGTH: 1347
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Nucleic acid sequence of CJ_LP_F6P4E

<400> SEQUENCE: 8

atgcaaacct cgacggcgta cgtgaggcag gtcatttggg gtcaagggac gagggacccc     60

cgcggcatct actcggtctg taccgcagac cccctcgtcc ttcgggccgc cctcaagcag    120

gcggtggagg atggctcccc cgcgctgatc gaggcgacgt ccaaccaggt gaaccagttc    180

ggcgggtata cggggatgga gccccggcg ttcgtggagt tcgtgctggg acttgcccgc    240

gagatgggac tcccgcccga gcggctgatc ctcgggggcg atcacctcgg ccccaaccca    300

tggcagcggc tggcggccga agaggccatg cggcatgcct gcgacctcgt cgaggccttc    360

gtggcctgcg gcttcaccaa gattcacctg gacgccagca tgcccctggg ggaggaacgg    420

gcaggcggtg cgctttcgaa acgggtggtg gccgaacgga ccgcccagct ctgcgaggcg    480

gccgaggcgg ccttcaggaa gcggtcccag gcggaggggg cgtcggcgcc tccgctctac    540

gtcatcggct ccgacgtgcc tccgcccggc ggcgagacct ccgggagcca ggggcccaag    600

gtgaccacgc cggaggagtt cgaggagacg gtcgcgctga cgcgggcgac ctttcacgat    660

cggggcctgg acgacgcctg gggacgggtg atcgccgtgg tggtccagcc gggggtggac    720

ttcggcgagt ggcaggttca cccctacgat cgggccgccg cggcgagcct tacccgagcc    780

ttgacgcagc atccggggct ggccttcgaa gggcactcca ccgactacca gacgccgggg    840

cggcttcgcc agatggcgga agacggcatc gccatcctga aggtggggcc ggccctcacc    900

ttcgccaagc gggaagcgct cttcgccctg aacgccctgg agtccgaagt gctggggacg    960

gacggccgag cacggcgctc caacgtcgaa gccgccctcg aagaggcgat gctcgccgat   1020

ccccgtcact ggagcgccta ctacagcggg gacgagcacg agctccgtct caagcggaag   1080

tacggcctct ccgaccggtg tcgctactac tggcccgtcc cttcggtgca ggaggccgtc   1140

cagcgcctcc ttggcaacct gcgcgaggcg gggatcccct tgcccctgct gagccagttc   1200

ctgccgcgcc agtacgagcg ggtgcgggag ggcgtcctgc gcaacgaccc ggaggagctg   1260
```

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gtcctggacc ggattcgtga cgtgttgcgg ggatatgcgg cggccgtggg gacgggcgct 1320

aggcgggcgg agccatcacc cgcgtga 1347

<210> SEQ ID NO 9
<211> LENGTH: 426
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Fructose-6-phosphate C4 epimerase derived from Thermanaerothrix daxensis (TD1)

<400> SEQUENCE: 9

Met Val Thr Tyr Leu Asp Phe Val Val Leu Ser His Arg Phe Arg Arg
1 5 10 15

Pro Leu Gly Ile Thr Ser Val Cys Ser Ala His Pro Tyr Val Ile Glu
20 25 30

Ala Ala Leu Arg Asn Gly Met Met Thr His Thr Pro Val Leu Ile Glu
35 40 45

Ala Thr Cys Asn Gln Val Asn Gln Tyr Gly Gly Tyr Thr Gly Met Thr
50 55 60

Pro Ala Asp Phe Val Arg Tyr Val Glu Asn Ile Ala Ala Arg Val Gly
65 70 75 80

Ser Pro Arg Glu Asn Leu Leu Leu Gly Gly Asp His Leu Gly Pro Leu
85 90 95

Val Trp Ala His Glu Pro Ala Glu Ser Ala Met Glu Lys Ala Arg Ala
100 105 110

Leu Val Lys Ala Tyr Val Glu Ala Gly Phe Arg Lys Ile His Leu Asp
115 120 125

Cys Ser Met Pro Cys Ala Asp Asp Arg Asp Phe Ser Pro Lys Val Ile
130 135 140

Ala Glu Arg Ala Ala Glu Leu Ala Gln Val Ala Glu Ser Thr Cys Asp
145 150 155 160

Val Met Gly Leu Pro Leu Pro Asn Tyr Val Ile Gly Thr Glu Val Pro
165 170 175

Pro Ala Gly Gly Ala Lys Ala Glu Ala Glu Thr Leu Arg Val Thr Arg
180 185 190

Pro Glu Asp Ala Ala Glu Thr Ile Ala Leu Thr Arg Ala Ala Phe Phe
195 200 205

Lys Arg Gly Leu Glu Ser Ala Trp Glu Arg Val Val Ala Leu Val Val
210 215 220

Gln Pro Gly Val Glu Phe Gly Asp His Gln Ile His Val Tyr Arg Arg
225 230 235 240

Glu Glu Ala Gln Ala Leu Ser Arg Phe Ile Glu Ser Gln Pro Gly Leu
245 250 255

Val Tyr Glu Ala His Ser Thr Asp Tyr Gln Pro Arg Asp Ala Leu Arg
260 265 270

Ala Leu Val Glu Asp His Phe Ala Ile Leu Lys Val Gly Pro Ala Leu
275 280 285

Thr Phe Ala Phe Arg Glu Ala Val Phe Ala Leu Ala Ser Ile Glu Asp
290 295 300

Trp Val Cys Asp Ser Pro Ser Arg Ile Leu Glu Val Leu Glu Thr Thr
305 310 315 320

Met Leu Ala Asn Pro Val Tyr Trp Gln Lys Tyr Tyr Leu Gly Asp Glu
325 330 335

Arg Ala Arg Arg Ile Ala Arg Gly Tyr Ser Phe Ser Asp Arg Ile Arg

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```
            340                 345                 350

Tyr Tyr Trp Ser Ala Pro Ala Val Glu Gln Ala Phe Glu Arg Leu Arg
        355                 360                 365

Ala Asn Leu Asn Arg Val Ser Ile Pro Leu Val Leu Leu Ser Gln Tyr
    370                 375                 380

Leu Pro Asp Gln Tyr Arg Lys Val Arg Asp Gly Arg Leu Pro Asn Gln
385                 390                 395                 400

Phe Asp Ala Leu Ile Leu Asp Lys Ile Gln Ala Val Leu Glu Asp Tyr
                405                 410                 415

Asn Val Ala Cys Gly Val Arg Ile Gly Glu
            420                 425
```

<210> SEQ ID NO 10
<211> LENGTH: 1281
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: DNA sequence of fructose-6-phosphate C4 epimerase derived fromThermanaerothrix daxensis (TD1)

<400> SEQUENCE: 10

```
atggttacct atttggattt tgtggtgctt tctcatcgtt ttaggcgccc cctgggcatt     60

acctcagtgt gttcggcgca tccgtatgtc attgaggcgg cgctgcgtaa tgggatgatg    120

acccatacac cggtcctaat cgaggccact tgcaatcaag tcaatcagta tgggggatat    180

acggggatga ccccggcaga tttcgtgcgg tatgtggaga atattgctgc acgggtaggc    240

tctccacgtg aaaacctcct tttgggtggc gatcatttgg gacccctggt ctgggctcat    300

gaacctgctg agagtgccat ggaaaaagct cgagctctgg tcaaagccta tgtagaggct    360

ggttttcgca aaattcatct ggattgctca atgccctgtg cggatgatcg cgatttttct    420

ccaaaggtca ttgctgagcg ggcagccgaa ttggctcagg tggcagagtc aacttgtgat    480

gttatgggct tgcccttgcc caactacgtc attggaaccg aggtgccccc agcaggtggc    540

gccaaggctg aagccgaaac tttgagggta acccgtccgg aggatgcagc ggagaccatt    600

gcactgacca gagcggcttt tttcaagcga ggtttagagt ctgcctggga acgtgtagtg    660

gcgttagtag tgcaacccgg tgttgaattc ggagatcatc agattcatgt ttaccgccgt    720

gaggaagcgc aggctctttc ccgcttcatt gaaagccagc ccggcttagt ctatgaggct    780

cactccaccg actatcagcc ccgtgatgcg ctgcgggctt tggttgagga tcatttcgca    840

atcctgaagg tgggtccggc gctaaccttt gcttttcgtg aggcagtttt tgccctggcc    900

agtatcgagg attgggtatg cgattcaccc agtcgcatcc tggaagtttt ggaaacaacc    960

atgctggcca acccggtcta ctggcaaaag tattacttgg gcgatgagcg agcgcgtcgg   1020

attgccagag ggtatagttt cagcgatcgc attcgttatt attggagtgc accagcggtt   1080

gaacaggcct ttgaacgctt gcgggcaaat ctgaatcgtg tttcgatccc ccttgtcctt   1140

ctcagtcagt atttgccgga tcaatatcgc aaagtgcggg atggacggct gcctaaccag   1200

tttgatgctt tgattctgga taaaatccaa gccgtactgg aagactacaa tgtggcgtgt   1260

ggtgtgagga taggggagtg a                                             1281
```

<210> SEQ ID NO 11
<211> LENGTH: 263
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Tagatose-6-phosphate phosphatase derived from

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Thermotoga maritima(T4)

<400> SEQUENCE: 11

Met Glu Gly Gly Ile Glu Leu Asp Arg Leu Asp Phe Ser Ile Lys Leu
1 5 10 15

Leu Arg Arg Val Gly His Phe Leu Met Leu His Trp Gly Lys Val Asp
20 25 30

Ser Val Glu Lys Lys Thr Gly Phe Lys Asp Ile Val Thr Glu Ile Asp
35 40 45

Lys Lys Ala Gln Glu Met Ile Val Glu Glu Ile Arg Lys Val Phe Pro
50 55 60

Asp Glu Asn Ile Ile Ala Glu Glu Gly Ile Ser Glu Asn Gly Lys Lys
65 70 75 80

Leu Trp Ile Ile Asp Pro Ile Asp Gly Thr Ile Asn Phe Val His Gly
85 90 95

Leu Pro Asn Phe Ser Ile Ser Ile Ala Tyr Val Glu Asn Gly Glu Val
100 105 110

Lys Met Gly Val Val His Ala Pro Ala Leu Asn Glu Thr Leu Tyr Ala
115 120 125

Glu Glu Asn Gly Gly Ala Phe Leu Asn Gly Glu Arg Ile Arg Val Ser
130 135 140

Gly Asn Thr Ser Leu Glu Glu Cys Val Gly Ser Thr Gly Ser Tyr Val
145 150 155 160

Asp Phe Thr Gly Lys Phe Ile Glu Lys Met Glu Lys Lys Thr Arg Arg
165 170 175

Val Arg Ile Leu Gly Ser Ala Ala Leu Asn Ala Cys Tyr Val Gly Ala
180 185 190

Gly Arg Val Asp Phe Phe Val Thr Trp Arg Ile Asn Pro Trp Asp Ile
195 200 205

Ala Ala Gly Leu Ile Val Val Lys Glu Ala Gly Gly Thr Val Thr Asp
210 215 220

Phe Ala Gly Lys Glu Ala Asn Val Phe Ser Lys Asn Phe Val Phe Ser
225 230 235 240

Asn Gly Leu Val His Glu Glu Val Leu Glu Val Val Asn Glu Val Leu
245 250 255

Lys Glu Ile Gly Glu Gly Lys
260

<210> SEQ ID NO 12
<211> LENGTH: 792
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: DNA sequence of tagatose-6-phosphate phosphatase derived from Thermotoga maritima (T4)

<400> SEQUENCE: 12

atggagggag ggatcgaatt ggacagactg gacttttcga taaaactcct gagaagggtt 60

gggcactttc tcatgcttca ctggggaaag gtggacagtg tggagaaaaa gaccggtttc 120

aaagacatcg tgacggaaat agacaaaaag gcccaggaga tgatagtgga ggagatcaga 180

aaggtttttc cggatgagaa cataatagcg gaggagggaa tctcggagaa cggaaaaaaa 240

ctctggataa tagatcccat agacgggacg ataaacttcg ttcatggact tcccaacttt 300

tccatctcca tcgcttacgt ggagaatgga gaggtgaaga tgggagttgt gcacgctcct 360

gcactcaacg aaacactcta cgccgaagaa aacgggggtg ctttttttgaa cggtgaaagg 420

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atcagggtgt ctggaaacac aagtcttgaa gagtgcgtgg gatcaacggg aagctatgtg 480

gatttcaccg gaaagtttat cgagaagatg gaaaagaaaa caaggagagt gagaattctg 540

gggagtgcgg cgctgaacgc ctgctacgtg ggagcaggga gggtggattt cttcgtcact 600

tggaggatca atccgtggga catcgcagca ggcctgatag ttgtgaaaga ggcgggagga 660

acggtgacag attttgccgg aaaagaggca aacgttttct cgaagaattt tgtcttctcc 720

aacggactcg ttcacgaaga agttctcgaa gtggtgaacg aggttctgaa agagatagga 780

gaggggaagt ga 792

<210> SEQ ID NO 13
<211> LENGTH: 451
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: glucose-6-phosphate isomerase derived from Thermotoga maritima(TN1)

<400> SEQUENCE: 13

Met Lys Lys Met Ala Leu Lys Phe Asp Phe Ser Asn Leu Phe Glu Pro
1 5 10 15

Asn Ile Ser Gly Gly Leu Arg Glu Glu Asp Leu Glu Ser Thr Lys Glu
20 25 30

Lys Val Ile Glu Ala Ile Lys Asn Phe Thr Glu Asn Thr Pro Asp Phe
35 40 45

Ala Arg Leu Asp Arg Lys Trp Ile Asp Ser Val Lys Glu Leu Glu Glu
50 55 60

Trp Val Val Asn Phe Asp Thr Val Val Val Leu Gly Ile Gly Gly Ser
65 70 75 80

Gly Leu Gly Asn Leu Ala Leu His Tyr Ser Leu Arg Pro Leu Asn Trp
85 90 95

Asn Glu Met Ser Arg Glu Glu Arg Asn Gly Tyr Ala Arg Val Phe Val
100 105 110

Val Asp Asn Val Asp Pro Asp Leu Met Ala Ser Val Leu Asp Arg Ile
115 120 125

Asp Leu Lys Thr Thr Leu Phe Asn Val Ile Ser Lys Ser Gly Ser Thr
130 135 140

Ala Glu Val Met Ala Asn Tyr Ser Ile Ala Arg Gly Ile Leu Glu Ala
145 150 155 160

Asn Gly Leu Asp Pro Lys Glu His Ile Leu Ile Thr Thr Asp Pro Glu
165 170 175

Lys Gly Phe Leu Arg Lys Val Val Lys Glu Glu Gly Phe Arg Ser Leu
180 185 190

Glu Val Pro Pro Gly Val Gly Gly Arg Phe Ser Val Leu Thr Pro Val
195 200 205

Gly Leu Phe Ser Ala Met Ala Glu Gly Ile Asp Ile Glu Glu Leu His
210 215 220

Asp Gly Ala Arg Asp Ala Phe Glu Arg Cys Lys Lys Glu Asp Leu Phe
225 230 235 240

Glu Asn Pro Ala Ala Met Ile Ala Leu Thr His Tyr Leu Tyr Leu Lys
245 250 255

Arg Gly Lys Ser Ile Ser Val Met Met Ala Tyr Ser Asn Arg Met Thr
260 265 270

Tyr Leu Val Asp Trp Tyr Arg Gln Leu Trp Ala Glu Ser Leu Gly Lys
275 280 285

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Arg Tyr Asn Leu Lys Gly Glu Glu Val Phe Thr Gly Gln Thr Pro Val
    290                 295                 300

Lys Ala Ile Gly Ala Thr Asp Gln His Ser Gln Ile Gln Leu Tyr Asn
305                 310                 315                 320

Glu Gly Pro Asn Asp Lys Val Ile Thr Phe Leu Arg Leu Glu Asn Phe
                325                 330                 335

Asp Arg Glu Ile Ile Ile Pro Asp Thr Gly Arg Glu Glu Leu Lys Tyr
            340                 345                 350

Leu Ala Arg Lys Arg Leu Ser Glu Leu Leu Leu Ala Glu Gln Thr Gly
        355                 360                 365

Thr Glu Glu Ala Leu Arg Lys Asn Asp Arg Pro Asn Met Lys Val Ile
    370                 375                 380

Phe Asp Arg Leu Thr Ser Tyr Asn Val Gly Gln Phe Phe Ala Tyr Tyr
385                 390                 395                 400

Glu Ala Ala Thr Ala Phe Met Gly Tyr Leu Leu Glu Ile Asn Pro Phe
                405                 410                 415

Asp Gln Pro Gly Val Glu Leu Gly Lys Lys Ile Thr Phe Ala Leu Met
            420                 425                 430

Gly Arg Glu Gly Tyr Glu Tyr Glu Ile Lys Asp Arg Thr Lys Lys Val
        435                 440                 445

Ile Ile Glu
    450
```

<210> SEQ ID NO 14
<211> LENGTH: 1356
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: DNA sequence of glucose-6-phosphate isomerase derived from Thermotoga maritima (TN1)

<400> SEQUENCE: 14

```
atgaaaaaga tggctttgaa atttgatttt tcaaatcttt ttgaaccgaa catctccggt     60

ggactgagag aggaagatct ggaaagcaca aaagaaaagg tgatagaggc gataaagaat    120

ttcactgaga acacaccgga ttttgccaga ctggacagaa aatggatcga ttcggtgaag    180

gaactcgagg agtgggtggt gaacttcgac acggtggtcg ttctgggaat tgggggatcc    240

ggtcttggaa accttgccct tcattattcg ttgagaccac tgaactggaa cgagatgtcg    300

agagaggaaa gaaacggtta tgcgagagtc ttcgtggtgg acaacgtaga tcccgatctc    360

atggcctccg tccttgatag gatagatctg aagacaacgc tgttcaacgt gatctcaaaa    420

tctggatcca cggctgaggt tatggcgaat tactcgatcg caaggggaat cctggaggct    480

aatggtctgg acccgaaaga acacatcctc atcacaacag atccagagaa gggctttttg    540

agaaaagtag tgaaagaaga gggcttcaga agtcttgagg tccctcccgg cgttggagga    600

aggttcagcg tgctgacgcc cgttggcctc ttctctgcca tggcggaggg tatcgacata    660

gaagaactcc acgacggtgc ccgggatgcg ttcgagagat gcaagaagga agacctgttc    720

gaaaatccag cggcgatgat cgccctcaca cactatctct atctgaagag aggaaagagc    780

atctccgtca tgatggccta ctccaacagg atgacctacc tcgtggactg gtacagacag    840

ctgtgggcag aaagtctggg aaagagatac aacctgaaag gagaggaggt cttcacgggt    900

cagaccccgg tgaaggcaat aggagccacc gatcagcact ctcagataca gctttacaac    960

gagggcccaa acgacaaagt gataacgttt ttgcggttgg aaaacttcga tagagagatc   1020
```

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ataataccgg acaccggaag agaagagctc aaataccttg caagaaaaag actctctgaa   1080

cttctccttg cagaacagac aggaacagag gaagccctaa ggaaaaacga cagaccgaac   1140

atgaaggtga tcttcgacag actcacctct tacaatgtgg gccagttctt cgcttattat   1200

gaagccgcaa ctgctttcat ggggtatctc ctcgagatca acccgtttga tcagccgggt   1260

gtggaacttg gaaagaagat cacgtttgcc ctcatgggaa gggaaggtta cgaatacgaa   1320

ataaaagatc gcaccaagaa ggtgatcata gaatga                             1356


<210> SEQ ID NO 15
<211> LENGTH: 471
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Phosphoglucomutase derived from Thermotoga
      neapolitana (CT2)

<400> SEQUENCE: 15

Met Ile Leu Phe Gly Thr Gly Gly Ile Arg Gly Val Met Arg Lys Gly
1               5                   10                  15

Glu Phe Asp Glu Asp Thr Val Lys Arg Ala Ser Leu Ser Val Ala Phe
            20                  25                  30

Trp Met Lys Gln Arg Lys Leu Lys Ser Val Val Ile Ala Tyr Asp Thr
        35                  40                  45

Arg Lys Asn Ser Arg Glu Phe Ala Glu Leu Ala Gly Arg Val Phe Ala
    50                  55                  60

Gly Glu Gly Ile Glu Ala Tyr Val Phe Pro Glu Pro Thr Pro Thr Pro
65                  70                  75                  80

Val Leu Ser Phe Ala Val Arg His Met Lys Ala Gly Ala Gly Val Val
                85                  90                  95

Ile Thr Ala Ser His Asn Pro Pro Glu Tyr Asn Gly Tyr Lys Val Tyr
            100                 105                 110

Thr Trp Asp Gly Val Gln Ala Ile Pro Glu Tyr Thr Asp Glu Ile Thr
        115                 120                 125

Glu Ile Tyr Lys Lys Val Asp Ile Ser Gly Val Arg Glu Gly Gly Phe
    130                 135                 140

Lys His Val Pro Ser Glu Val Lys Glu Ser Tyr Ile Glu Lys Val Val
145                 150                 155                 160

Glu Ile Val Ser Asn Leu Pro Arg Arg Thr Asp Leu Asp Val Ala Tyr
                165                 170                 175

Ser Pro Leu His Gly Thr Gly Ala Asn Tyr Val Pro Glu Val Leu Arg
            180                 185                 190

Arg Leu Gly Phe Lys Val Arg Pro Val Glu Glu Gln Met Lys Pro Asp
        195                 200                 205

Pro Asn Phe Ser Thr Val Pro Thr Pro Asn Pro Glu Glu Asp Glu Ala
    210                 215                 220

Leu Val Leu Leu Asn Lys Lys Glu Ala Thr Leu Gly Leu Ala Thr Asp
225                 230                 235                 240

Pro Asp Cys Asp Arg Val Gly Val Val Tyr Arg Gly Arg Arg Leu Thr
                245                 250                 255

Gly Asn Gln Val Gly Val Leu Leu Thr Asp Phe Leu Leu Glu His Val
            260                 265                 270

Lys Val Glu Asn Pro Leu Val Ile Lys Thr Ile Val Thr Thr Asp Met
        275                 280                 285

Val Arg Pro Ile Cys Glu Glu Arg Gly Ala Tyr Leu Glu Glu Thr Pro
    290                 295                 300
```

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```
Thr Gly Phe Lys Phe Ile Gly His Leu Ile Glu Glu His Thr Lys Lys
305                 310                 315                 320

Gly Asp Arg Asn Phe Val Phe Gly Phe Glu Glu Ser Cys Gly Tyr Leu
                325                 330                 335

Ala Gly Asp His Ala Arg Asp Lys Asp Gly Val Val Gly Ser Val Leu
            340                 345                 350

Ser Ala Ile Ala Phe Ser Asn Tyr Asp Pro Tyr Glu Lys Leu Glu Glu
        355                 360                 365

Leu Tyr Arg Lys Tyr Gly Tyr Tyr Met Glu Lys Leu Ile Asn Phe Lys
    370                 375                 380

Phe Glu Asp Val Ser Lys Ala Ile Glu Ile Tyr Asn Ser Leu Lys Glu
385                 390                 395                 400

Tyr Asp Gly Ile Ile Asp Tyr Ser Arg Gly Tyr Lys Gly Ile Ile Pro
                405                 410                 415

Asn Glu Thr Ile Ala Phe Val Phe Glu Lys Ser Arg Ile Phe Val Arg
            420                 425                 430

Pro Ser Gly Thr Glu Pro Lys Leu Lys Val Tyr Ile His Val Arg Gly
        435                 440                 445

Asp Thr Arg Glu Glu Ser Glu Asn Leu Met Lys Glu Ser Glu Arg Lys
    450                 455                 460

Ile Arg Glu Ile Leu Lys Leu
465                 470


<210> SEQ ID NO 16
<211> LENGTH: 1416
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: DNA sequence of phosphoglucomutase derived from
      Thermotoga neapolitana (CT2)

<400> SEQUENCE: 16

atgatcctgt ttggaacggg tggaattcga ggtgtgatga gaaagggaga gttcgatgag     60

gacacggtga agagggcttc actgagcgtc gccttctgga tgaaacagag aaaactgaaa    120

agcgttgtga tcgcctacga cacgagaaaa aactccagag agttcgcaga gcttgccgga    180

agggtcttcg caggtgaagg aatagaagcc tacgtgtttc cagaaccaac gccaacaccg    240

gttctctctt tcgcagtgag gcacatgaag gccggtgccg gtgttgtcat aacagcgagt    300

cacaatcctc cagaatacaa cggatacaag gtttacacct gggatggcgt tcaggcaata    360

ccagagtaca cggacgagat caccgaaata tacaaaaagg tcgatatctc cggagtgagg    420

gagggaggtt tcaaacacgt accttccgag gtgaaggaga gttacataga gaaagtggtt    480

gagatagtct cgaaccttcc aagaagaacg gaccttgacg ttgcctactc tccactccat    540

ggaacgggag caaactatgt tccggaggtt ttgagaagac tcggtttcaa agtgagacct    600

gtggaagaac agatgaaacc cgatccaaac ttctccacag tcccaactcc aaatcccgaa    660

gaagatgaag cgctcgtttt gctgaacaaa aaggaagcga cccttggact tgcaaccgac    720

ccggactgcg acagggtggg agtggtgtac agaggaagaa ggctcacagg aaaccaggtt    780

ggagtgctcc ttacggactt tctcctcgaa cacgtgaagg tagaaaaccc tctcgtgata    840

aaaacgatcg tcaccacgga catggtgagg cccatctgtg aggaaagggg tgcctatctc    900

gaagaaacac caacaggttt caaattcatc ggtcatttga tagaagaaca cacaaagaaa    960

ggtgacagaa acttcgtctt tggtttcgag gaaagctgtg gatacctcgc aggagaccac   1020
```

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gcaagggaca aagatggtgt tgtgggaagt gtcctctctg cgatagcctt cagcaactac 1080

gacccgtacg aaaaactcga agaactctac agaaagtacg gttactacat ggaaaaactc 1140

atcaacttca agttcgaaga cgtcagcaaa gcgatagaaa tatacaactc cctgaaagag 1200

tacgatggaa taatcgatta ctccagaggt tacaaaggaa taattccaaa cgaaaccata 1260

gccttcgtgt tcgaaaaatc cagaatcttc gtcagaccat ctggaacaga accgaagctc 1320

aaggtgtaca tccacgtgag aggggacaca agggaagagt cagagaatct gatgaaggaa 1380

agtgaaagaa agatcaggga gatcctgaaa ctgtga 1416

<210> SEQ ID NO 17
<211> LENGTH: 270
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Polyphosphate-dependent glucokinase CJ dg ppgk

<400> SEQUENCE: 17

Met Leu Ala Ala Ser Asp Ser Ser Gln His Gly Gly Lys Ala Val Thr
1 5 10 15

Leu Ser Pro Met Ser Val Ile Leu Gly Ile Asp Ile Gly Gly Ser Gly
20 25 30

Ile Lys Gly Ala Pro Val Asp Thr Ala Thr Gly Lys Leu Val Ala Glu
35 40 45

Arg His Arg Ile Pro Thr Pro Glu Gly Ala His Pro Asp Ala Val Lys
50 55 60

Asp Val Val Val Glu Leu Val Arg His Phe Gly His Ala Gly Pro Val
65 70 75 80

Gly Ile Thr Phe Pro Gly Ile Val Gln His Gly His Thr Leu Ser Ala
85 90 95

Ala Asn Val Asp Lys Ala Trp Ile Gly Leu Asp Ala Asp Thr Leu Phe
100 105 110

Thr Glu Ala Thr Gly Arg Asp Val Thr Val Ile Asn Asp Ala Asp Ala
115 120 125

Ala Gly Leu Ala Glu Ala Arg Phe Gly Ala Gly Ala Gly Val Pro Gly
130 135 140

Glu Val Leu Leu Leu Thr Phe Gly Thr Gly Ile Gly Ser Ala Leu Ile
145 150 155 160

Tyr Asn Gly Val Leu Val Pro Asn Thr Glu Phe Gly His Leu Tyr Leu
165 170 175

Lys Gly Asp Lys His Ala Glu Thr Trp Ala Ser Asp Arg Ala Arg Glu
180 185 190

Gln Gly Asp Leu Asn Trp Lys Gln Trp Ala Lys Arg Val Ser Arg Tyr
195 200 205

Leu Gln Tyr Leu Glu Gly Leu Phe Ser Pro Asp Leu Phe Ile Ile Gly
210 215 220

Gly Gly Val Ser Lys Lys Ala Asp Lys Trp Gln Pro His Val Ala Thr
225 230 235 240

Thr Arg Thr Arg Leu Val Pro Ala Ala Leu Gln Asn Glu Ala Gly Ile
245 250 255

Val Gly Ala Ala Met Val Ala Ala Gln Arg Ser Gln Gly Asp
260 265 270

<210> SEQ ID NO 18
<211> LENGTH: 813
<212> TYPE: DNA

-continued

<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: DNA sequence of polyphosphate-dependent glucokinase CJ dg ppgk

<400> SEQUENCE: 18

atgctggcag ccagtgacag cagccagcat ggcgggaagg ctgttacgct atctcccatg 60

agcgtgatcc tcgggattga cataggtggg agcggcatca agggggcccc tgtggacacg 120

gcaaccggga agctggtggc cgagcgccac cgcatcccca cgcccgaggg cgcgcaccca 180

gacgcggtga aggacgtggt ggttgagctg gtgcggcatt ttgggcatgc ggggccagtc 240

ggcatcactt tccctggcat cgtgcagcac ggccataccc tgagcgcagc caatgtggat 300

aaagcctgga ttggcctgga cgccgacacg ctttttactg aggcgaccgg tcgcgacgtg 360

accgtgatca acgacgcaga tgccgcgggg ctagcggagg cgaggttcgg ggccggggca 420

ggtgtgccgg gcgaggtgtt gctgttgacc tttgggacag gcatcggcag cgcgctgatc 480

tataacggcg tgctggtgcc caacaccgag tttgggcatc tgtatctcaa gggcgacaag 540

cacgccgaga catgggcgtc cgaccgggcc cgtgagcagg gcgacctgaa ctggaagcag 600

tgggccaaac gggtcagccg gtacctccag tatctggaag gtctcttcag tcccgatctc 660

tttatcatcg gtgggggcgt gagcaagaag gccgacaagt ggcagccgca cgtcgcaaca 720

acacgtaccc gcctggtgcc cgctgccctc cagaacgagg ccggaatcgt gggggccgcg 780

atggtggcgg cgcagcggtc acaggggac taa 813

<210> SEQ ID NO 19
<211> LENGTH: 253
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Polyphosphate-dependent glucokinase CJ at ppgk

<400> SEQUENCE: 19

Met Gly Arg Gln Gly Met Glu Ile Leu Gly Ile Asp Ile Gly Gly Ser
1 5 10 15

Gly Ile Lys Gly Ala Pro Val Asp Val Glu Thr Gly Gln Leu Thr Ala
20 25 30

Glu Arg Tyr Arg Leu Pro Thr Pro Glu Asn Ala Leu Pro Glu Glu Val
35 40 45

Ala Leu Val Val Ala Gln Ile Val Glu His Phe Gln Trp Lys Gly Arg
50 55 60

Val Gly Ala Gly Phe Pro Ala Ala Ile Lys His Gly Val Ala Gln Thr
65 70 75 80

Ala Ala Asn Ile His Pro Thr Trp Ile Gly Leu His Ala Gly Asn Leu
85 90 95

Phe Ser Glu Lys Cys Gly Cys Pro Val Ser Val Leu Asn Asp Ala Asp
100 105 110

Ala Ala Gly Leu Ala Glu Met Ile Phe Gly Ala Gly Lys Gly Gln Lys
115 120 125

Gly Val Val Leu Met Ile Thr Ile Gly Thr Gly Ile Gly Thr Ala Leu
130 135 140

Phe Thr Asp Gly Ile Leu Val Pro Asn Thr Glu Leu Gly His Ile Glu
145 150 155 160

Ile Arg Gly Lys Asp Ala Glu Gln Arg Ser Ser Glu Ala Ala Arg Gln
165 170 175

Arg Lys Asp Trp Thr Trp Gln Gln Trp Ala Lys Arg Leu Asn Glu His

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```
            180                 185                 190

Leu Glu Arg Leu Glu Ala Leu Phe Trp Pro Asp Leu Phe Ile Leu Gly
        195                 200                 205

Gly Gly Ala Val Lys Asn His Glu Lys Phe Phe Pro Tyr Leu Lys Leu
    210                 215                 220

Arg Thr Pro Phe Val Ala Ala Lys Leu Gly Asn Leu Ala Gly Ile Val
225                 230                 235                 240

Gly Ala Ala Trp Tyr Ala His Thr Gln Glu Thr Gln Ala
                245                 250
```

<210> SEQ ID NO 20
<211> LENGTH: 762
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: DNA sequence of polyphosphate-dependent glucokinase CJ at ppgk

<400> SEQUENCE: 20

```
atggggaggc agggcatgga aattttaggg attgatatcg gaggatccgg catcaaaggg     60

gctccggtgg atgtagaaac cggccagtta accgccgagc gataccgctt acccaccccc    120

gaaaatgcct tacctgaaga agtggctctg gtagttgccc aaattgtcga acactttcag    180

tggaaaggtc gtgtaggggc aggatttcct gctgccatca agcacggcgt ggcacagacg    240

gccgcaaaca tccaccctac atggattgga cttcatgctg gcaacctttt cagcgaaaaa    300

tgcggatgtc ctgtctcagt gttgaatgat gcggatgctg ccggactggc ggaaatgatc    360

tttggggcag gaaaaggcca gaaaggggtg gtgctgatga ttaccattgg cactggcatc    420

gggacagccc tgttcaccga tgggatattg gtccctaata ccgagttggg acatattgaa    480

attcggggca aagatgccga acagcgctct tcggaagccg cccgccagcg gaaggattgg    540

acctggcaac aatgggcaaa gcgtctgaat gagcatttgg agcgcctgga agccctgttc    600

tggcccgatt tattcatcct tggtggaggg gcagtaaaaa atcatgaaaa gttcttccct    660

tatctaaaac tgcgtactcc ctttgttgca gcaaaattgg ggaatctggc tgggattgta    720

ggcgcagcgt ggtatgctca cacccaggaa acgcaagcct ga                       762
```

<210> SEQ ID NO 21
<211> LENGTH: 823
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: a-glucanphosphorylase derived from Thermotoga neapolitana (CT1)

<400> SEQUENCE: 21

```
Met Leu Lys Lys Leu Pro Glu Asn Leu Glu His Leu Glu Glu Leu Ala
1               5                   10                  15

Tyr Asn Leu Trp Trp Ser Trp Ser Arg Pro Ala Gln Arg Leu Trp Arg
            20                  25                  30

Lys Ile Asp Pro Glu Gly Trp Glu Glu His Arg Asn Pro Val Lys Ile
        35                  40                  45

Leu Lys Glu Val Ser Asp Glu Arg Leu Glu Glu Leu Ser Lys Asp Asp
    50                  55                  60

Asp Phe Ile Ser Leu Tyr Glu Leu Thr Ile Glu Arg Phe Lys Asp Tyr
65                  70                  75                  80

Met Glu Lys Glu Asp Thr Trp Phe Asn Val Asn Tyr Pro Glu Trp Asp
                85                  90                  95
```

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Glu Lys Ile Val Tyr Met Cys Met Glu Tyr Gly Leu Thr Lys Ala Leu
            100                 105                 110

Pro Ile Tyr Ser Gly Gly Leu Gly Ile Leu Ala Gly Asp His Leu Lys
        115                 120                 125

Ser Ala Ser Asp Leu Gly Leu Pro Leu Ile Ala Ile Gly Leu Leu Tyr
    130                 135                 140

Lys His Gly Tyr Phe Thr Gln Gln Ile Asp Arg Asp Gly Lys Gln Ile
145                 150                 155                 160

Glu Ile Phe Pro Asp Tyr Asn Pro Glu Asp Leu Pro Met Lys Pro Leu
                165                 170                 175

Lys Asp Glu Lys Gly Asn Gln Val Ile Val Glu Val Pro Leu Asp Ser
            180                 185                 190

Thr Val Val Lys Ala Arg Val Phe Glu Val Lys Val Gly Arg Val Ser
        195                 200                 205

Leu Tyr Leu Leu Asp Pro Asp Ile Glu Glu Asn Glu Glu Arg Tyr Arg
    210                 215                 220

Lys Ile Cys Asn Tyr Leu Tyr Asn Pro Glu Pro Asp Val Arg Val Ser
225                 230                 235                 240

Gln Glu Ile Leu Leu Gly Ile Gly Gly Met Lys Leu Leu Arg Ala Leu
                245                 250                 255

Asn Leu Lys Pro Gly Val Ile His Leu Asn Glu Gly His Pro Ala Phe
            260                 265                 270

Ser Ser Leu Glu Arg Ile Lys Asn Tyr Met Glu Glu Gly Tyr Ser Phe
        275                 280                 285

Thr Glu Ala Leu Glu Ile Val Arg Gln Thr Ser Val Phe Thr Thr His
    290                 295                 300

Thr Pro Val Pro Ala Gly His Asp Arg Phe Pro Phe Asp Leu Val Glu
305                 310                 315                 320

Lys Lys Leu Ser Lys Phe Phe Glu Gly Phe Glu Lys Arg Asn Leu Leu
                325                 330                 335

Met Asp Leu Gly Lys Asp Glu Thr Gly Ser Phe Asn Met Thr Tyr Leu
            340                 345                 350

Ala Leu Arg Thr Ser Ser Phe Ile Asn Gly Val Ser Lys Leu His Ala
        355                 360                 365

Glu Val Ser Arg Arg Met Phe Lys Asn Val Trp Gln Gly Val Pro Val
    370                 375                 380

Glu Glu Ile Pro Ile Glu Gly Ile Thr Asn Gly Val His Met Gly Thr
385                 390                 395                 400

Trp Ile Asn Arg Glu Met Arg Lys Leu Tyr Asp Arg Tyr Leu Gly Arg
                405                 410                 415

Val Trp Arg Asp His Thr Asp Leu Glu Gly Ile Trp Tyr Gly Val Asp
            420                 425                 430

Arg Ile Pro Asp Glu Glu Leu Trp Gln Ala His Leu Arg Ala Lys Lys
        435                 440                 445

Arg Phe Ile Glu Tyr Ile Lys Glu Ser Val Arg Arg Arg Asn Glu Arg
    450                 455                 460

Leu Gly Ile Asp Glu Asp Val Pro Asn Ile Asp Glu Asn Ser Leu Ile
465                 470                 475                 480

Ile Gly Phe Ala Arg Arg Phe Ala Thr Tyr Lys Arg Ala Val Leu Leu
                485                 490                 495

Leu Ser Asp Leu Glu Arg Leu Lys Lys Ile Leu Asn Asp Pro Glu Arg
            500                 505                 510
```

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```
Pro Val Tyr Val Val Tyr Ala Gly Lys Ala His Pro Arg Asp Asp Ala
        515                 520                 525

Gly Lys Glu Phe Leu Lys Arg Ile Tyr Glu Val Ser Gln Met Pro Glu
    530                 535                 540

Phe Lys Asn Arg Ile Ile Val Leu Glu Asn Tyr Asp Ile Gly Met Ala
545                 550                 555                 560

Arg Leu Met Val Ser Gly Val Asp Val Trp Leu Asn Asn Pro Arg Arg
                565                 570                 575

Pro Met Glu Ala Ser Gly Thr Ser Gly Met Lys Ala Ala Ala Asn Gly
            580                 585                 590

Val Leu Asn Ala Ser Val Tyr Asp Gly Trp Trp Val Glu Gly Tyr Asn
        595                 600                 605

Gly Arg Asn Gly Trp Val Ile Gly Asp Glu Ser Val Leu Pro Glu Thr
    610                 615                 620

Glu Val Asp Asp Pro Arg Asp Ala Glu Ala Leu Tyr Asp Leu Leu Glu
625                 630                 635                 640

Asn Glu Ile Ile Pro Thr Tyr Tyr Glu Asn Lys Glu Lys Trp Ile Phe
                645                 650                 655

Met Met Lys Glu Ser Ile Lys Ser Val Ala Pro Arg Phe Ser Thr Thr
            660                 665                 670

Arg Met Leu Lys Glu Tyr Thr Glu Lys Phe Tyr Ile Lys Gly Leu Val
        675                 680                 685

Asn Lys Glu Trp Leu Glu Arg Lys Glu Asn Ala Glu Arg Phe Gly Ala
    690                 695                 700

Trp Lys Glu Arg Ile Leu Arg Asn Trp Ser Ser Val Ser Ile Glu Arg
705                 710                 715                 720

Ile Val Leu Glu Asp Thr Arg Ser Val Glu Val Thr Val Lys Leu Gly
                725                 730                 735

Asp Leu Ser Pro Asp Asp Val Leu Val Glu Leu Leu Ile Gly Arg Gly
            740                 745                 750

Glu Ser Met Glu Asp Leu Glu Ile Trp Lys Val Ile Gln Ile Arg Lys
        755                 760                 765

His Arg Arg Glu Gly Asp Leu Phe Ile Tyr Ser Tyr Val Asn Gly Ala
    770                 775                 780

Leu Gly His Leu Gly Ser Pro Gly Trp Phe Tyr Ala Val Arg Val Leu
785                 790                 795                 800

Pro Tyr His Pro Lys Leu Pro Thr Arg Phe Leu Pro Glu Ile Pro Val
                805                 810                 815

Val Trp Lys Lys Val Leu Gly
            820


<210> SEQ ID NO 22
<211> LENGTH: 2472
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: DNA sequence of a-glucanphosphorylase derived
      from Thermotoga neapolitana (CT1)

<400> SEQUENCE: 22

atgctgaaga aactcccgga gaatctggag catctggaag aactcgccta caacctctgg     60

tggagctggt ctaggcccgc tcagagactc tggagaaaga tagatccgga aggctgggag    120

gaacacagaa accccgttaa aatactgaaa gaagtttctg atgaaaggct cgaagaactt    180

tcaaaagatg atgatttcat atccctctac gaactcacca ttgaaaggtt caaggattac    240
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atggagaaag aagacacctg gttcaacgtg aactaccccg aatgggacga gaagatcgtc 300

tacatgtgta tggagtacgg tttgaccaaa gcccttccga tctactccgg tggtcttgga 360

atcctcgcgg gagaccatct caaatccgca agcgatcttg gacttcctct catagcgatc 420

ggacttctct acaaacatgg atatttcacc cagcagatcg acagagatgg aaaacagata 480

gagattttcc ctgattacaa cccagaggac ttacccatga agcccctgaa ggatgaaaag 540

ggaaaccagg tgatcgtgga ggttcctctc gacagtaccg tggtgaaggc acgtgttttt 600

gaagtgaagg taggaagggt gagtctgtac ctgctcgatc cggacatcga ggaaaacgag 660

gaacgataca gaaagatctg caactacctt tacaacccgg aacccgatgt gagggtctcc 720

caggagatac tcctcggaat tgggggaatg aagcttctca gggctctgaa cctgaaacca 780

ggagtcatcc atctgaacga aggacatccg gcgttctctt ccctcgaaag gataaagaac 840

tacatggaag aaggatattc cttcacagag gcccttgaga tcgtgagaca gacgagtgtg 900

tttacaaccc acacacccgt tcccgctgga cacgacagat ttccctttga cctcgtggaa 960

aagaaacttt cgaaattctt cgaaggattc gaaaagagaa atcttctcat ggatcttggg 1020

aaagatgaaa caggcagttt caacatgacg tatcttgccc tgagaacgtc ctctttcata 1080

aacggcgtga gcaaactgca tgcggaagtt tccagaagga tgttcaaaaa cgtgtggcag 1140

ggtgttcccg tggaggaaat accgatcgaa gggataacga acggcgttca catgggaacc 1200

tggatcaacc gtgagatgag aaaactgtac gacagatatc tcggaagggt atggagagat 1260

cacaccgacc ttgagggtat ctggtacggt gttgacagga ttccagatga agaactctgg 1320

caggctcacc tgagggcaaa gaagagattc atcgagtaca taaaagaatc ggtaagaaga 1380

agaaacgaga gactgggaat cgacgaagat gtgccgaaca tcgatgaaaa ttcgctcatc 1440

ataggttttg caagaaggtt tgccacttac aagagggcag ttctcctgct cagcgatctg 1500

gagagactca agaagatcct caacgatcca gaaagacccg tttacgtggt ctatgcgggg 1560

aaggcccatc caagggacga tgcggggaag gaatttttga aacgcatcta cgaagtctcg 1620

cagatgcctg agttcaaaaa caggatcatc gtactggaaa actacgacat tggaatggca 1680

cggctcatgg tgtcgggagt ggatgtgtgg ctgaacaacc cgagaagacc catggaagca 1740

agtggaacaa gcggaatgaa ggcagcagcc aacggagttc ttaacgcgag tgtttacgat 1800

ggatggtggg ttgaagggta caacggcaga aacggctggg tcataggcga tgaaagcgtt 1860

cttccagaga cggaagtgga cgatcccagg gacgcagaag cactctacga tctcctcgaa 1920

aacgaaatca tcccaaccta ctacgaaaac aaagaaaagt ggatcttcat gatgaaagag 1980

agcataaaga gtgttgctcc aagattcagc accaccagaa tgctcaaaga atacacggag 2040

aagttctaca taaagggact tgtgaacaaa gaatggcttg aaagaaaaga aaacgccgaa 2100

aggtttggtg catggaagga aaggatcctc agaaactgga gcagcgtttc catagaaaga 2160

atcgtccttg aggacacaag gagtgttgag gtgacggtga aactgggaga cctttcacct 2220

gatgatgtac tggttgaact tttgattgga agaggagaaa gcatggaaga tctggagatc 2280

tggaaggtga tacagataag aaagcacaga agggaagggg atctgttcat ctacagttat 2340

gtcaacggtg ccctcggtca tcttggctct ccgggatggt tctacgcggt gagggtgcta 2400

ccttatcatc cgaaacttcc caccagattc ttgccggaga tacctgtggt gtggaaaaag 2460

gttctcgggt ga 2472

<210> SEQ ID NO 23
<211> LENGTH: 441

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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 4-a-glucanotransferase derived from Thermotoga maritima(TN2)

<400> SEQUENCE: 23

Met Ile Gly Tyr Gln Ile Tyr Val Arg Ser Phe Arg Asp Gly Asn Phe
1 5 10 15

Asp Gly Val Gly Asp Phe Lys Gly Leu Lys Gly Ala Ile Ser Tyr Leu
20 25 30

Lys Glu Leu Gly Val Asp Phe Val Trp Leu Met Pro Val Phe Ser Ser
35 40 45

Ile Ser Phe His Gly Tyr Asp Val Val Asp Phe Tyr Ser Phe Lys Ala
50 55 60

Glu Tyr Gly Asp Glu Lys Asp Phe Arg Glu Met Ile Glu Ala Phe His
65 70 75 80

Asp Asn Gly Ile Lys Val Val Leu Asp Leu Pro Ile His His Thr Gly
85 90 95

Phe Leu His Val Trp Phe Gln Lys Ala Leu Lys Gly Asp Pro His Tyr
100 105 110

Arg Asp Tyr Tyr Val Trp Ala Ser Glu Lys Thr Asp Leu Asp Glu Arg
115 120 125

Arg Glu Trp Asp Asn Glu Arg Ile Trp His Pro Leu Glu Asp Gly Arg
130 135 140

Phe Tyr Arg Gly Leu Phe Gly Pro Leu Ser Pro Asp Leu Asn Tyr Asp
145 150 155 160

Asn Pro Gln Val Phe Glu Glu Met Lys Lys Val Val Tyr His Leu Leu
165 170 175

Glu Met Gly Val Asp Gly Phe Arg Phe Asp Ala Ala Lys His Met Arg
180 185 190

Asp Thr Leu Glu Gln Asn Val Arg Phe Trp Arg Tyr Phe Leu Ser Asp
195 200 205

Ile Glu Gly Ile Phe Leu Ala Glu Ile Trp Ala Glu Ser Lys Val Val
210 215 220

Asp Glu His Gly Arg Ile Phe Gly Tyr Met Leu Asn Phe Asp Thr Ser
225 230 235 240

His Cys Ile Lys Glu Ala Val Trp Lys Glu Asn Phe Lys Val Leu Ile
245 250 255

Glu Ser Ile Glu Arg Ala Leu Val Gly Lys Asp Tyr Leu Pro Val Asn
260 265 270

Phe Thr Ser Asn His Asp Met Ser Arg Leu Ala Ser Phe Glu Gly Gly
275 280 285

Leu Ser Glu Glu Lys Val Lys Leu Ser Leu Ser Ile Leu Phe Thr Leu
290 295 300

Pro Gly Val Pro Leu Ile Phe Tyr Gly Asp Glu Leu Gly Met Lys Gly
305 310 315 320

Ile Tyr Arg Lys Pro Asn Thr Glu Val Val Leu Asp Pro Phe Pro Trp
325 330 335

Ser Glu Asn Met Cys Val Glu Gly Gln Thr Phe Trp Lys Trp Pro Ala
340 345 350

Tyr Asn Asp Pro Phe Ser Gly Val Ser Val Glu Tyr Gln Arg Arg Asn
355 360 365

Arg Asp Ser Ile Leu Ser His Thr Met Arg Trp Ala Gly Phe Arg Gly
370 375 380

-continued

```
Glu Asn His Trp Leu Asp Arg Ala Asn Ile Glu Phe Leu Cys Lys Glu
385                 390                 395                 400

Glu Lys Leu Leu Val Tyr Arg Leu Val Asp Glu Gly Arg Ser Leu Lys
                405                 410                 415

Val Ile His Asn Leu Ser Asn Gly Glu Met Val Phe Glu Gly Val Arg
            420                 425                 430

Val Gln Pro Tyr Ser Thr Glu Val Val
        435                 440
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<210> SEQ ID NO 24
<211> LENGTH: 1326
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: DNA sequence of 4-a-glucanotransferase derived from Thermotoga maritima(TN2)

<400> SEQUENCE: 24

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atgataggct accagatcta cgtgagatca ttcagggatg gaaacttcga tggtgtgggg     60

gatttcaaag gattgaaagg tgcgatttcc tacctgaaag aactgggtgt tgattttgtc    120

tggctcatgc ccgtcttttc ctccatttcc ttccacgggt atgacgtggt ggatttttat    180

tctttcaaag ccgagtacgg agacgagaaa gactttagag agatgatcga ggcgttccac    240

gacaacggta taaaagtcgt tctcgatctt cccatccatc atactggttt cctccatgtg    300

tggtttcaga aagccctgaa aggagatcca cactacaggg attattacgt atgggcgagt    360

gaaaaaacgg atctggacga aagaagagag tgggacaacg aaaggatctg gcatcctctg    420

gaggacggaa ggttctacag aggacttttc ggtcccctct cacccgatct gaactacgat    480

aacccgcagg tttttgaaga gatgaagaag gtggtttatc accttcttga aatgggagtg    540

gacggattca gattcgacgc agcaaagcac atgagagata ctctggaaca gaacgttcgc    600

ttttggaggt atttcctctc cgatattgag ggaatattcc ttgcggaaat ctgggcagaa    660

tccaaagttg tggatgaaca cggcaggata ttcggctaca tgctaaattt cgatacctca    720

cactgtatta aggaagcggt gtggaaggaa aacttcaaag tgttgatcga gtcgatcgaa    780

agggccctgg ttggaaaaga ttatctgccg gtgaacttca catcgaacca tgatatgtca    840

aggcttgcga gtttcgaagg agggttgagt gaagagaagg tgaaactctc actttccatt    900

ctgttcacgc ttcccggggt tcctctcata ttctacggag acgaactggg aatgaaagga    960

atctatcgaa aaccgaacac ggaagtcgtg ctggatccgt tcccctggag cgaaaacatg   1020

tgtgttgaag gccagacatt ttggaaatgg cccgcgtata acgatccatt ctccggtgtt   1080

tctgttgagt atcagaggag aaatcgtgat tcgattctct cacacacgat gaggtgggca   1140

ggattcagag gggaaaatca ctggctggac agggcaaaca tcgaatttct gtgcaaagaa   1200

gaaaaactgc tcgtgtacag actggtcgat gaagggcgtt ctctgaaagt gatacacaac   1260

ctgtcgaatg gtgaaatggt gtttgaggga gtgcgcgtac aaccctacag cacggaggtg   1320

gtttga                                                              1326
```

<210> SEQ ID NO 25
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Forward primer DNA sequence of CT1

<400> SEQUENCE: 25

aggagaaact catatgctga agaaactccc ggag 34

<210> SEQ ID NO 26
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Reverse primer DNA sequence of CT1

<400> SEQUENCE: 26

agccccctcg agcccgagaa c 21

<210> SEQ ID NO 27
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Forward primer DNA sequence of CT2

<400> SEQUENCE: 27

aaagggcata tgatcctgtt tggaac 26

<210> SEQ ID NO 28
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Reverse primer DNA sequence of CT2

<400> SEQUENCE: 28

ataccagtct cgagcagttt caggatc 27

<210> SEQ ID NO 29
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Forward primer DNA sequence of TN1

<400> SEQUENCE: 29

ttactgaggg catatgaaaa agatggcttt gaaa 34

<210> SEQ ID NO 30
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Reverse primer DNA sequence of TN1

<400> SEQUENCE: 30

aagacgcgtc gacttctatg atcaccttct 30

<210> SEQ ID NO 31
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Forward primer DNA sequence of TN2

<400> SEQUENCE: 31

atagagggca tatgataggc taccagatct a 31

<210> SEQ ID NO 32
<211> LENGTH: 24
<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Reverse primer DNA sequence of TN2

<400> SEQUENCE: 32

atcttcatct cgagaaccac ctcc 24

<210> SEQ ID NO 33
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Forward primer DNA sequence of T4

<400> SEQUENCE: 33

ttttcatatg gagggaggga tcgaattg 28

<210> SEQ ID NO 34
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Reverse primer DNA sequence of T4

<400> SEQUENCE: 34

catactcgag cttcccctct cctatct 27

What is claimed is:

1. A method of producing tagatose, comprising:

a) producing tagatose-6-phosphate by contacting fructose-6-phosphate with tagatose-bisphosphate aldolase, a microorganism expressing the tagatose-bisphosphate aldolase, or a culture of the microorganism; and

b) producing tagatose by converting tagatose-6-phosphate produced in Step a) into tagatose.

2. The method of claim **1**, wherein the Step b) further comprises contacting the tagatose-6-phosphate produced in Step a) with tagatose-6-phosphate phosphatase, a microorganism expressing the tagatose-6-phosphate phosphatase, or a culture of the microorganism.

3. The method of claim **1**, wherein prior to producing the tagatose-6-phosphate, the method further comprises producing fructose-6-phosphate by converting glucose-6-phosphate into fructose-6-phosphate by contacting glucose-6-phosphate with glucose-6-phosphate-isomerase, a microorganism expressing the glucose-6-phosphate-isomerase, or a culture of the microorganism.

4. The method of claim **3**, wherein prior to producing the fructose-6-phosphate, the method further comprising comprises producing glucose-6-phosphate by converting glucose-1-phosphate into glucose-6-phosphate by contacting glucose-1-phosphate with phosphoglucomutase, a microorganism expressing the phosphoglucomutase, or a culture of the microorganism.

5. The method of claim **4**, wherein prior to producing the glucose-6-phosphate, the method further comprises producing glucose-1-phosphate by converting starch, maltodextrin, or sucrose into glucose-1-phosphate by contacting starch, maltodextrin, sucrose, or a combination thereof with (i) α-glucan phosphorylase, starch phosphorylase, maltodextrin phosphorylase, or sucrose phosphorylase; (ii) a microorganism expressing the α-glucan phosphorylase, starch phosphorylase, maltodextrin phosphorylase, or sucrose phosphorylase; or (iii) a culture of the microorganism.

6. The method of claim **3**, wherein prior to producing the fructose-6-phosphate, the method further comprises producing glucose-6-phosphate by converting glucose into glucose-6-phosphate by contacting glucose with glucokinase, a microorganism expressing the glucokinase, or a culture of the microorganism.

7. The method of claim **6**, wherein prior to producing the glucose-6-phosphate, the method further comprises producing glucose by converting starch, maltodextrin or sucrose into glucose by contacting starch, maltodextrin, sucrose, or a combination thereof with α-amylase, pullulanase, glucoamylase, sucrase, or isoamylase; a microorganism expressing the α-amylase, pullulanase, glucoamylase, sucrase, or isoamylase; or a culture of the microorganism.

8. The method of claim **1**, wherein the contacting is performed at pH 5.0 to 9.0, 40° C. to 80° C., and/or for 0.5 hours to 24 hours.

9. The method of claim **1**, wherein the tagatose-bisphosphate aldolase consists of an amino acid sequence of SEQ ID NO: 1, 3, 5, or 7.

10. A method of producing tagatose, comprising:

contacting (a) starch, maltodextrin, sucrose, or a combination thereof with (b) (i) tagatose-6-phosphate phosphatase, (ii) tagatose-bisphosphate aldolase, (iii) glucose-6-phosphate-isomerase, (iv) phosphoglucomutase or glucokinase, (v) phosphorylase, and (vi) one or more of α-amylase, pullulanase, isoamylase, glucoamylase, or sucrase; and (c) phosphate,

wherein the tagatose-bisphosphate aldolase has activity to convert fructose-6-phosphate into tagatose-6-phosphate.

11. The method of claim **2**, wherein the tagatose-6-phosphate phosphatase consists of the amino acid sequence of SEQ ID NO: 11.

* * * * *